SYSTEMIC INFLAMMATION

in Preterm Infants

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“Sit down before the fact as a little child,
be prepared to give up every preconceived notion,
follow humbly wherever and to whatever abysses nature leads,
or you shall learn nothing.”

Thomas Henry Huxley (1825-1895)

ABSTRACT

Even though mortality among preterm infants has decreased, their risk for chronic complications such as bronchopulmonary dysplasia (BPD) and neurological disability remains significant. One common risk factor for these is exposure to inflammation. The fetus may be exposed prenatally during maternal chorioamnionitis. Pre-eclampsia is also associated with low-grade maternal inflammation. Postnatally, local and systemic inflammation is present during respiratory distress syndrome (RDS). Furthermore, septic infections in the preterm infant are an important source of inflammatory stimuli and can lead to death in only a few hours. The diagnosis of septic infection is difficult, since reliable diagnostic markers are unavailable.

This thesis evaluates peri- and postnatal systemic inflammation in preterm infants in septic infections, in RDS treated with mechanical ventilation and surfactant treatment, and in preterm infants prenatally exposed to chorioamnionitis and pre-eclampsia. Surface expressions of the activation markers CD11b, CD54, and CD62L, determined by flow cytometry on circulating phagocytes and T lymphocytes, serve as indicators of systemic inflammation.

The main findings: I) In preterm infants with developing late-onset sepsis and fulminant necrotizing enterocolitis, a significant increase in CD11b expression on circulating phagocytes is already present on the day of onset of clinical symptoms. II) In preterm infants with RDS, circulating phagocytes become activated within hours after start of mechanical ventilation. In preterm infants treated for RDS with nasal continuous positive airway pressure, no such activation occurs. III) In preterm infants, RDS is associated during the first days of life with fewer circulating helper and cytotoxic T lymphocytes, of which the greater proportions are activated. Even greater proportions of circulating T cells are activated in infants subsequently developing BPD. IV) In preterm infants born after maternal pre-eclampsia, RDS-associated phagocyte CD11b up-regulation is greater than in preterm infants not exposed to pre-eclampsia during the first week of life.

These findings suggest that I) an increase in CD11b expression on circulating phagocytes can identify preterm infants with late-onset sepsis as early as at sampling for blood culture and may thus aid in the diagnosis. II) In preterm infants with RDS, initiation of mechanical ventilation, but not the use of nasal continuous positive airway pressure, promotes a systemic inflammatory reaction; exogenous surfactant does not seem to promote inflammation. III) In addition to activation of circulating cells of the innate immunity in preterm infants with RDS, the circulating cells of the adaptive immunity are activated. The activation of adaptive immunity may link acute inflammation and development of chronic inflammation-associated problems such as BPD. IV) Maternal pre-eclampsia may prime neonatal immunity to react more strongly to postnatal stimuli.

In conclusion, the preterm infant is exposed to numerous potentially injurious events such as intrauterine inflammation, respiratory distress syndrome (RDS), and systemic infections, all evoking systemic inflammation. Due to ongoing development of the lung and the brain, this may, in addition to acute injury, lead to aberrant lung and brain development and to clinical syndromes of BPD and neurologic sequelae.

ABSTRACT IN FINNISH

Keskosten tehohoidon kehityksen myötä keskosten kuolleisuus on vähentynyt, mutta pitkäaikaissairauksien, kuten kroonisen keuhkosairauden, bronkopulmonaalisen dysplasian (BPD), ja neurologisten ongelmien, esiintyvyys on pienimmillä keskosilla merkittävää. Yhteinen näille altistava tekijä on altistuminen tulehdukselle. Ennen syntymää äidin kohtutulehdus voi altistaa sikiön tulehdukselle. Myös pre-eklampsiaan eli raskausmyrkytykseen liittyy äidin yleistynyt tulehdus. Syntymän jälkeen keskosen hengitysvaikeusoireyhtymään (respiratory distress syndrome, RDS) liittyy sekä keuhkojen paikallinen että koko elimistön yleistynyt tulehdus. Tärkeitä tulehdusta aiheuttavia tekijöitä keskosilla ovat myös infektiot. Infektiodiagnostiikka keskosella on luotettavien diagnostisten testien puuttuessa haasteellista, ja tauti voi edetä kuolemaan tunneissa.

Tämä väitöskirja arvioi syntymänjälkeistä yleistynyttä tulehdusta keskosilla yleistyneessä infektiossa; surfaktantti- ja hengityskonehoidetussa RDS-taudissa; sekä äidin pre-eklampsian vaikutusta keskosen tulehdukseen. Yleistynyttä tulehdusta arvioidaan mittaamalla virtaussytometrilla aktivaatiomerkki-aineina toimivien adheesiomolekyylien CD11b, CD54 ja CD62L määrää veren fagosyyttien ja T lymfosyyttien pinnalla.

Tärkeimmät löydökset: I) Keskosella, jolla on kehittyvä infektio tai nekrotisoiva enterokoliitti, kiertävien fagosyyttien CD11b on merkitsevästi koholla jo ensimmäisten kliinisten oireiden ilmaantuessa; II) RDS:aa sairastavalla keskosella veren fagosyytit aktivoituvat jo ennen surfaktantin antoa hengityskonehoidon aloittamiseen liittyen. Nasaaliylipaineessa hoidetulla RDS:aa sairastavalla keskosella aktivaatiota ei tapahdu. III) Keskosella RDS:aan liittyy matalammat määrät kiertäviä T-soluja, ja näistä suurempi osuus on aktivoituneita. Aktivoituneiden solujen osuus on suurempi keskosilla, jotka kehittävät BPD:n. IV) Pre-eklampsiaraskaudesta syntyneillä keskosilla RDS:aan liittyvä fagosyyttien CD11b-määrän nousu ensimmäisen elinviikon aikana on suurempaa kuin verrokeilla, jotka eivät ole altistuneet pre-eklampsialle.

Löydöksistä voidaan päätellä, että I) kiertävien fagosyyttien CD11b-määrän nousu tunnistaa kehittyvän infektion keskosella jo ensimmäisten kliinisten oireiden ilmaantuessa ja voi siten auttaa diagnoosin tekemisessä; II) RDS:aa sairastavalla keskosella hengitys-konehoidon aloittaminen, mutta ei nasaaliylipaineen käyttö, aiheuttaa yleistyneen tulehdusreaktion. Surfaktantin anto ei lisää tulehdusta RDS:aa sairastavalla keskosella; III) Synnynnäisen immuniteetin kiertävien solujen aktivaation lisäksi myös hankitun immuniteetin solut aktivoituvat RDS:aa sairastavalla keskosella. Hankinnaisen immuniteetin aktivaatio voi toimia välittävänä tekijänä akuutin tulehduksen ja kroonisten tulehdukseen liittyvien sairauksien, kuten BPD:n kehittymisen välillä; IV) Äidin pre-eklampsia voi herkistää vastasyntyneen immuniteettia reagoimaan voimakkaammin syntymänjälkeisiin ärsykkeisiin.

Yhteenvetona voidaan todeta, että keskonen voi altistua monille tulehdusta aiheuttaville tekijöille, kuten istukka- ja kohtutulehdukselle, pre-eklampsialle, RDS:lle ja infektioille. Keuhkojen ja aivojen kehityksen ollessa kesken tulehdus voi akuutin vaurion lisäksi johtaa myös normaalin kehityksen häiriintymiseen ja sitä kautta BPD:n ja neurologisten vaurioiden kehittymiseen.

TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following articles referred to in the text by Roman numerals I to IV:

I. Turunen R, Andersson S, Nupponen I, Kautiainen H, Siitonen S, Repo H. Increased CD11b-density on circulating phagocytes as an early sign of late-onset sepsis in extremely low-birth-weight infants. *Pediatr Res* 57:270-275, 2005

II. Turunen R, Nupponen I, Siitonen S, Repo H, Andersson S. Onset of mechanical ventilation is associated with rapid activation of circulating phagocytes in preterm infants. *Pediatrics* 117:448-454, 2006

III. Turunen R, Vaarala O, Nupponen I, Kajantie E, Siitonen S, Savolainen M, Lano A, Repo H, Andersson S. Activation of T cells in preterm infants with respiratory distress syndrome. *Neonatology*. 96:248-258, 2009

IV. Turunen R, Andersson S, Laivuori H, Kajantie E, Siitonen S, Repo H, Nupponen I. High postnatal inflammation in mechanically ventilated preterm infants born to mothers with early-onset pre-eclampsia. Submitted 2009

ABBREVIATIONS

APC	antigen-presenting cell
ARDS	acute respiratory distress syndrome
AUC	area under curve
BPD	bronchopulmonary dysplasia
BW	birth weight
CARS	compensatory anti-inflammatory reaction syndrome
CD	cluster of differentiation
CI	confidence interval
DC	dendritic cell
ELBW	extremely low birth weight (<1000g)
GA	gestational age
ICAM-1	inter-cellular adhesion molecule-1
IFN-γ	interferon γ
IL	interleukin
IUGR	intrauterine growth reduction
IVH	intraventricular hemorrhage
MHC	major histocompatibility complex
nCPAP	nasal continuous positive airway pressure
NK cell	natural killer cell
NPV	negative predictive value
PMN	polymorphonuclear cell
PPROM	preterm premature rupture of the membranes
PPV	positive predictive value
RDS	respiratory distress syndrome
ROC	receiver operator characteristic
SD	standard deviation
Tc cell	cytotoxic T cell
TGF	transforming growth factor
Th cell	helper T cell
Treg cell	regulatory T cell
TLR	toll-like receptor
TNF	tumor necrosis factor
VEGF	vascular endothelial growth factor
VLBW	very low birth weight (<1500g)

INTRODUCTION

Preterm birth, defined as birth at 37 gestational weeks or less, is the single most important cause of perinatal morbidity and mortality worldwide (McGormick 1985). In Finland the incidence of preterm birth has remained stable during the last two decades, being 5.7% in 2007 (National Birth Register 2008). In contrast, in many developed countries the incidence of preterm birth has risen, rather than fallen, over time, being 5 to 9% in Europe and 12 to 13% in USA (Slattery et al. 2002, Hamilton et al. 2007). About 5% of preterm births occur at less than 28 weeks' gestational age, 15% at 28 to 31 weeks, 20% at 32 to 33, and 60 to 70% at weeks 34 to 36 (National Birth Register 2007, Goldenberg et al. 2008).

The survival of infants born before the completion of 32 gestational weeks has increased due to improved practices in neonatal intensive care. However, these infants remain at high risk for potentially disabling chronic complications. The lungs and brain, unlike many other organs, continue to develop throughout the entire pregnancy. This renders these organs vulnerable to injury due to preterm birth, which results in high rates of chronic pulmonary and neurological problems in infants born extremely preterm (Saigal S et al. 2008, Palta et al. 2000, Kobaly et al. 2008, Doyle et al. 2006, Kilbride et al. 2003).

Inflammation is the host's reaction to invading pathogens, aimed at protecting the host from infection or as response to tissue injury contributing to repair and restoration of the normal functions of the tissue. It is tightly controlled, since inflammatory mechanisms possess a great injury potential. In the face of an overwhelming stimulus, such as in septic infection or massive tissue destruction, the inflammatory reaction may become systemic and threaten the host's survival. In addition, inadequate control may lead to chronic inflammation which can impair tissue function permanently due to fibrosis.

This thesis evaluates the systemic inflammatory reactions in preterm infants associated with antenatal factors, respiratory distress syndrome, and septic infection.

REVIEW OF THE LITERATURE

THE IMMUNE SYSTEM

The main tasks of the immune system are to prevent invasion of pathogenic micro-organisms into the body, to watch for non-organized cells, and to collaborate in the processes of repair after tissue destruction and clearance of cell debris. The immune system is complex and has overlapping systems to ensure its efficiency. It is composed of multiple cell types at distinct locations throughout the body. Parts of this system are ready at birth, i.e. innate, and they react similarly to repeated challenge. In contrast, the cells involved in adaptive immunity are able to enhance their reactions upon any new encounter with the same antigen, the phenomenon referred to as immunological memory. Even though traditionally classified as two separate systems, innate and adaptive immunity do not operate separately but rather are complementary and work concomitantly and in concert.

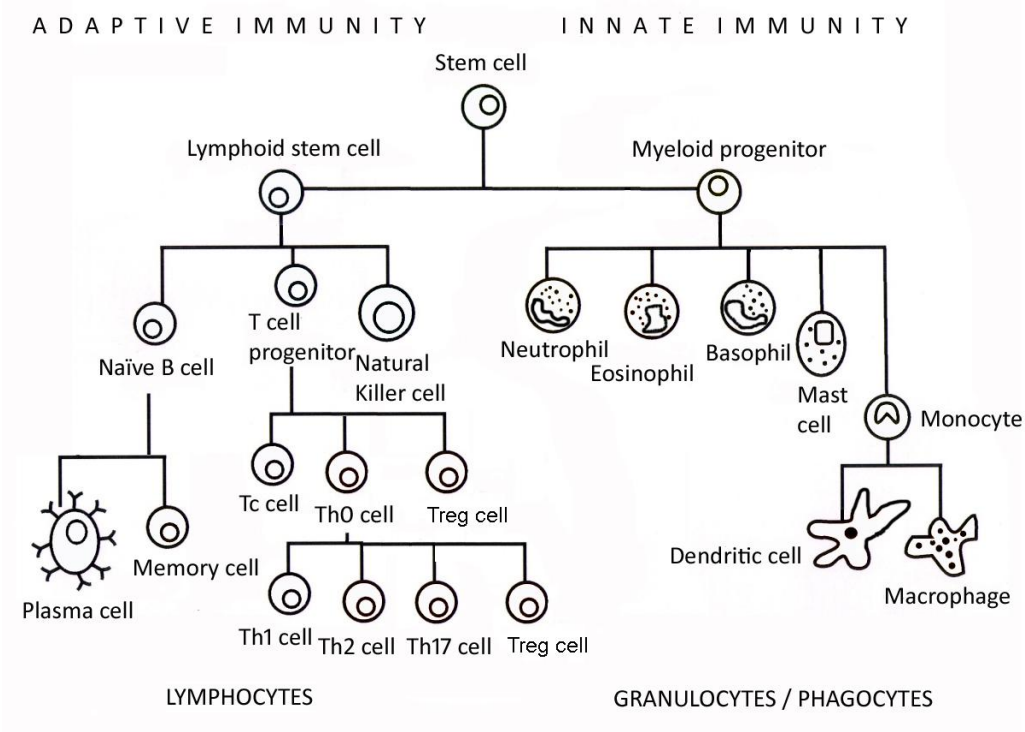

Figure 1 Simplified overview of the development of the cells of the immune system. Modified and reprinted with permission from *www.textbookofbacteriology.net.*

Innate immunity

The first line of defense against invading pathogens consists of the physiological, chemical, and microbial barriers of the human body. These include the skin, the mucosa, the bacterial normal flora, the epithelial cilia of the respiratory tract, and the chemical anti-microbial components of body secretions such as tears, saliva, urine, gastric acid, bile acid, and pancreatic enzymes. If these barriers fail, and pathogens gain entrance to the body, the cells and molecules of the innate immunity are the next to activate. These include neutrophils, monocytes, macrophages, dendritic cells, the complement system, and the acute phase proteins. Neutrophils, monocytes, and macrophages are also collectively called phagocytes due to their efficiency in phagocytosis.

The components of the innate immunity are ancient and are conserved among different species. Cells of the innate immunity recognize pathogens via a limited repertoire of non-antigen-specific pattern-recognition receptors such as Toll-like receptors (TLR). They can be found on cell surfaces (TLR1, 2, 4, 5, 6, 11) for detection of extra-cellular pathogens or in intracellular organelles (TLR3, 7, 8, 9) for intracellular pathogen detection (Miyake 2007, Lee 2007). Their ligands include microbial membrane components and other microbial products such as lipopolysaccharides, lipopeptides, and double-stranded RNA, collectively referred to as pathogen-associated molecular patterns (PAMPs), as well as endogenous danger signals released in states of tissue destruction or stress such as heat shock proteins, extracellular matrix-degradation products, β-defensin, and surfactant protein A (Vivier & Malissen 2005, Miyake 2007, Medzhitov 2008).

Neutrophils

Neutrophilic granulocytes (a.k.a. polymorphonuclear granulocytes) form the largest leukocyte population in the peripheral blood. They are short-lived, circulating for only a few hours, after which they leave the circulation to enter the tissues, live there another 24 hours and undergo programmed cell death, apoptosis. They are efficient phagocytic cells. The neutrophil's intracytoplasmic granules contain proteases (such as elastase, cathepsin G, protease 3), hydrolases, antibiotic proteins (bactericidal/permeability-increasing protein, α-defensins, serprocidins), and oxidants (hydrogen peroxide, hypophalites, chloramines), which are readily released upon neutrophil activation (Nathan 2002). The release of these potent molecules facilitates the killing of the invading pathogens and the recruitment of inflammatory cells to the site of inflammation by promoting extracellular matric breakdown. However, these functions underlie the potential of tissue destruction if the control of neutrophil activation is overriden.

Monocytes, macrophages, dendritic cells

Monocytes are circulating cells of myeloid progenitor origin. The majority of the monocyte-derived cells are within the tissues, where they form the population of tissue macrophages and myeloid dendritic cells (DC). Proinflammatory and immune stimuli may

result in further monocyte recruitment into peripheral tissues, where they differentiate into inflammatory monocytes (Yona & Gordon 2007).

Tissue-resident macrophages and DCs are the triggers of any inflammatory reaction. They recognize the molecular danger signals indicative of infection or tissue destruction, engulf and kill pathogens, secrete pro-inflammatory cytokines, and activate components of the complement to recruit circulating leukocytes and thus initiate the inflammatory reaction. DCs also engulf pathogens and express their antigens on their cell surface for lymphocyte recognition. Thus they are known also as antigen-presenting cells (APC).

Natural killer or NK cells

Natural killer (NK) cells develop from lymphocyte precursors, but exhibit features characteristic for cells of the innate immunity. They act by direct cellular cytotoxicity and by producing chemokines and cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor (TNF). NK cells have a complex repertoire of pathogen-recognizing receptors and are capable of modest expansion of the pathogen-specific subset. NK cells are important killers of pathogen-infected host cells and of tumor cells and also regulate the functions of other cells of both adaptive and innate immunity. In addition, cross-talk between DCs and NK cells is probably an important regulatory phenomenon of both cell types. Depending on the context, NK cell interaction may mature or kill immature DCs, and the cytokines produced by activated NK cells direct the Th cell maturation towards the type 1 response (Raulet 2004).

Mast cells, eosinophils, basophils

Mast cells are mainly tissue-reminiscent cells. They are the cells responsible for initiation of inflammation together with macrophages (Gurish & Boyce 2006, Prussin & Metcalfe 2006). Basophils share similarities with mast cells, but are, however, considered a separate cell line. Their role in immune defence and human disease is unclear, but, similar to eosinophils, they release mediators important in allergic reactions (Prussin & Metcalfe 2006).

Complement and acute phase proteins

In addition to effector cells, distinct soluble serum proteins play a role in innate immunity. The complement system is a complex set of proteins that circulates in an inactive form. Several stimuli, e.g., microbial proteins, lipids, and carbohydrate structures, antibodies bound to antigen, and C-reactive protein (CRP) can activate the complement system via different pathways. The effector products of the complement work together with the innate immunity by promoting inflammation. This may involve chemokines or anaphylatoxins and enhancement of microbial clearance by opsonizing bacteria, i.e., tagging them for recognition by neutrophils and macrophages, and by creating bacterial cell membrane-perforating complexes that result in cell lysis (Harboe &

Mollnes 2008). In addition, present in plasma are acute phase proteins involved in or formed by the initiation of the inflammatory response such as CRP, mannose-binding protein, LPS-binding protein, and soluble CD14.

Phagocyte migration to the site of inflammation

The process of migration of the circulating phagocyte to the site of inflammation is mediated by a series of different leukocyte-endothelium and leukocyte-extracellular matrix interactions mediated by distinct adhesion molecules. When the underlying tissue becomes inflamed, it releases chemokines that activate the endothelium to increase its expression of endothelial adhesion molecules such as sulphated sialyl Lewis-X, E-selectin, and inter-cellular-adhesion molecule-1 (ICAM-1, CD54). Chemoattractants, chemokines, and pro-inflammatory cytokines prime circulating leukocytes and make them responsive to further stimuli. As a result, the leukocyte starts rolling on the activated endothelium, mediated by loose selectin-oligosaccharide connections between the leukocyte and the endothelium, and this process slows the speed of the cells. If the inflammatory stimulus continues, the neutrophil is triggered to up-regulate its surface expression of integrins such as CD11bs that mediate the tight adhesion between the neutrophil and endothelium. The cell is then able to transmigrate through the vessel wall and start migrating towards the site of inflammation (Luster et al. 2005) (Figure 2).

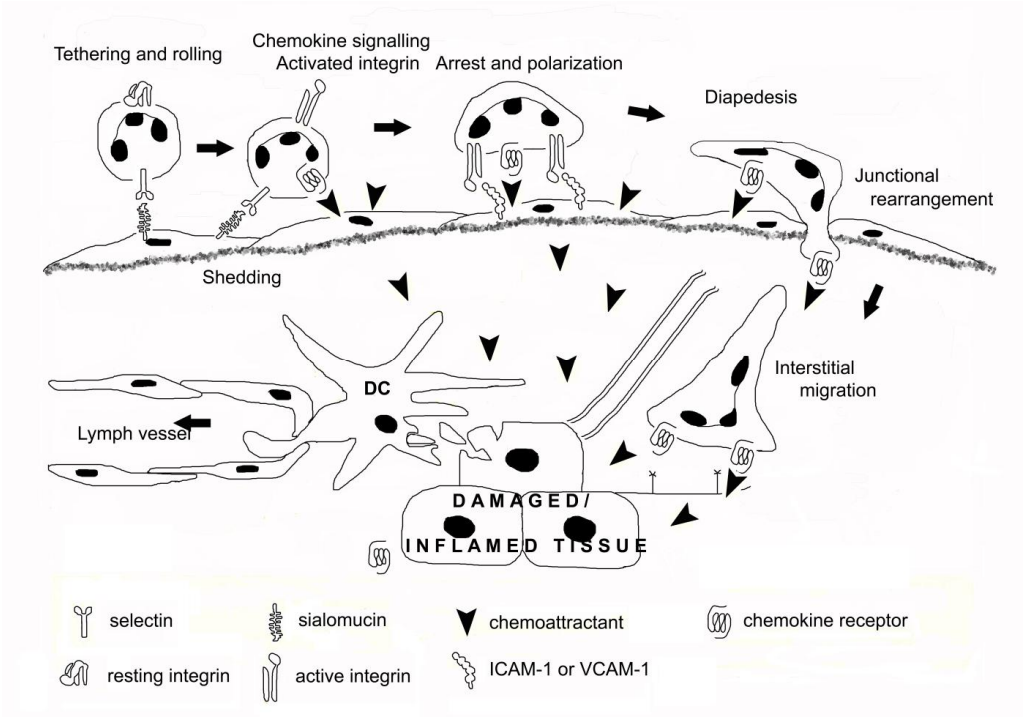

Figure 2 Inflammation due to tissue damage or infection induces the release of cytokines and inflammatory chemoattractants (arrowheads) from distressed stromal cells and macrophages. The inflammatory signals induce upregulation of endothelial adhesion molecules. Chemoattractants, particularly chemokines, are produced by or translocated across venular endothelial cells and are displayed to rolling leukocytes. Leukocytes undergo a multistep adhesion cascade and then migrate across the venular wall (diapedesis) through the endothelium and underlying basement membrane. Inflammatory signals also induce dendritic cells to undergo maturation, start processing material from damaged tissues and invading pathogens, and enter draining lymph vessels. In lymph nodes, these mature dendritic cells activate naive T cells, which enter the blood and migrate back to the site of inflammation. Modified from Luster et al. 2005 and reprinted with permission from the copyright holder, *Nature immunology*.

Adaptive immunity

The adaptive immune responses are antigen-specific reactions mediated by T- and B-lymphocytes. The key features of adaptive immunity are the ability to create antigen-specific responses, to respond with antigen-specific clonal cell expansion, and to create immunological memory.

T cells

T cells are considered effector cells of the adaptive immunity. They are further divided into CD4 expressing helper T cells (Th) and CD8 expressing cytotoxic T cells (Tc). Th cells are key players in initiating the adaptive immune response. Depending on the characteristics of the stimulus initiating the inflammatory response recognized by APCs, Th cells differentiate either to Th1, Th2, or Th17 cells with distinct functions, target cells, and operation patterns (Mosmann & Coffman 1989, Cua et al. 2003, Langrish et al. 2005, Veldhoen et al. 2006, Bettelli et al. 2008, Zhu & Paul 2008). Th1 cells direct the immune response towards a cytotoxic response by activating Tc cells and macrophages. Th2 cells activate a humoral, B cell-mediated immune response and antibody production, as well as activate eosinophils. Th17 cells work with innate immunity by helping neutrophils mount an efficient response against extra-cellular pathogens. In addition they appear to be potent inducers of autoimmunity (Korn et al. 2007, Stockinger et al. 2007, Bettelli et al. 2008). Regulatory T cells (Treg) modulate the reactions of other lymphocytes and regulate self tolerance (Zhu & Paul 2008).

Activated cytotoxic T cells are capable of inducing selective and organized cell death in other cells. This is demonstrated by induction of apoptosis in the virus infected host cell by the antigen-specific cytotoxic T cell's recognizing the viral antigens expressed by the infected cell.

T cell activation

T cell activation requires antigen presentation and co-stimulation provided by APCs (especially DCs, but also macrophages and B cells). The APCs present antigens on their surface on major histocompatibility complex (MHC) molecules or human leukocyte antigen (HLA) molecules in humans. The T cell screens these antigen-MHC complexes on the APCs with its T cell receptor (TCR) CD3 complex, and high affinity recognition is the prerequisite for T cell activation. After antigen recognition, co-stimulatory signals are essential for T cell survival, activation, proliferation, and maturation into effector cells. If this fails, the T cell that has recognized its own antigen but has not received co-stimulation becomes anergic, considered to be one mechanism of tolerization. Furthermore, sustained antigen signaling is required for the T cell to be committed to activation and proliferation (Lanzavecchia & Sallusto 2000).

ICAM-1 or CD54 is an adhesion molecule expressed on endothelial cells, epithelial cells, fibroblasts, T cells, B cells, dendritic cells, macrophages and eosinophils. Its ligands are CD11a/CD18 and CD11b/CD18, expressed only on leukocytes (Stanciu & Djukanovic 1998). On resting T cells its expression is low and is up-regulated in response to inflammatory cytokines (Hubbard & Rothlein 2000). CD54 expressed on T cells and other

leukocytes mediates cell-cell interactions in processes related to cell recognition and activation. CD54 provides a co-stimulatory signal to the T cell upon cell activation (Chirathaworn et al. 2002, Lanzavecchia & Sallusto 2000), and its expression is increased in activated cells (Stanciu & Djukanovic 1998, Hubbard & Rothlein 2000)

CD62L or L-sel is expressed on resting lymphocytes. Its physiological role is to direct the cell to lymphatic vessels. In naïve T cells, CD62L expression is high but decreases upon cell activation and maturation to memory cells (Hannet I et al. 1992, Stanciu & Djukanovic 1998).

B cells

B cells are responsible for humoral immune responses. Naïve B cells require specific antigen presentation and co-stimulation by Th cells. Activated B cells mature to plasma cells that account for antigen-specific antibody production. Antibodies act by binding to their specific antigens, e.g., on the surface of an invading pathogen, thereby enhancing phagocytic clearance or cytotoxic killing of the pathogen or hampering its invasion into the tissues by coating the bacterial surface.

Distinctive features of innate and adaptive immunity

The receptors of innate immunity are germline-encoded, and their distribution is subset-specific but nonclonal, whereas the antigen receptors of adaptive immunity are clonally distributed products of site-specific somatic recombination. The repertoire of the innate immunity is limited and selected in groups of individuals within a given species. The repertoire of the adaptive immunity is immense and selected in each individual within a given species. Furthermore, the adaptive immunity bears memory, in contrast to innate immunity (Vivier & Malissen 2005).

Interplay between innate and adaptive immunity

One of our immune system's astonishing features is its capacity to distinguish among the contexts in which the antigen is encountered. This is possible mainly due to the complex interactions of monocyte-derived APCs and lymphocytes. The APC first recognizes a harmful process or invading pathogen, identifies the type of injury through signaling from its different TLRs, presents the injury-associated antigens to T cells, and, in cases of recognition by the T cell, provides the co-stimulation necessary for T cell activation. Furthermore, the APC is, through secretion of different cytokine compositions, capable of directing the type of T cell response required for pathogen clearance or for repairing the tissue injury and healing (Lanzavecchia & Sallusto 2000).

INFLAMMATION

As described by Aulus Cornelius Celsus (ca 25 BCE - 50 CE) already by that time the macroscopic characteristics of inflammation were *rubor, tumor, dolor, calor,* or redness, swelling, pain, and heat. This description reflects the increased blood flow and increased fluid extravasation mediated by locally activated mast cells and neutrophils that release histamine, eikosanoids, tumor necrosis factor (TNF), cytokines, tryptases, proteases, and chemokines. Histologically, inflammation is characterized by accumulation of neutrophils, monocytes, and, in chronic inflammation, lymphocytes. This is accomplished by transmigration of circulating leukocytes through the activated endothelium, described in more detail above. The release of proteases, hydrolases, and oxidants by local inflammatory cells is aimed at hampering pathogen migration and proliferation and aiding leukocyte migration in the extracellular matrix. However, this process also promotes tissue breakdown. The main aim is to accomplish targeted destruction of the invading pathogen and assisted clearance and repair after succession of this mission (Nathan 2002).

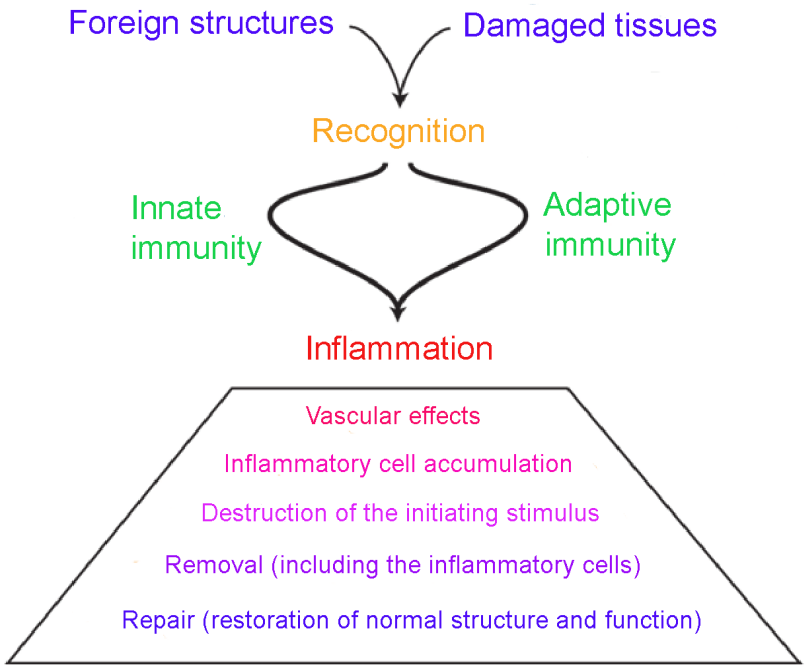

Figure 3 Foreign structures of microbial origin as well as products from damaged tissues are recognized by the innate and adaptive immunity. Both systems contribute to the development of inflammation, its augmentation, regulation, and, after removal of the inflammatory stimulus, restoration of the non-inflammatory state and repair of the tissue. Modified from Henson 2005 and reprinted with permission from the copyright holder, *Nature immunology.*

Control and resolution of inflammation

In healthy tissues, inflammatory stimuli are scarce. In addition, active mechanisms exist that suppress inappropriate activation of inflammation (Nathan 2002). However, when facing a true infectious threat, it is equally crucial to rapidly amplify the response even at the cost of collateral damage to the host. After eradication of the pathogen, the system must undergo rapid deactivation and begin repair.

Before becoming pro-inflammatory, the inflammatory cells use multiple checkpoints for reinforcement before their amplification and continuation of the inflammatory response. Many inflammatory cells require engagement with both pathogen- and host-cell signaling to become pro-inflammatory (Nathan 2002). First, neutrophils and mast cells require sustained pathogen stimulation or massive tissue stress to become pro-inflammatory and start recruiting other cell populations. Secondly, DCs require activating cytokines or direct cell-cell interactions with other immune cells and microbial products or products of necrotic host cells to become professional APCs. Furthermore, T cells need specific antigen recognition and sustained APC co-stimulation, and B cells need antigen recognition and T cell stimulation to become committed to maturation into effector cells, clonal expansion, and formation of memory cells (Nathan 2002, Serhan & Savill 2005).

It is suggested that an active, coordinated program of resolution of inflammation is initiated in the first few hours after an inflammatory response begins – the beginning programs the end. This is examplified by a built-in switch in production, from the pro-inflammatory eicosanoids, prostaglandins, that promote vessel dilatation and increased blood flow, to anti-inflammatory eicosanoids, lipoxin, resolvins, and protectins that reduce endothelial permeability and granulocyte extravasation and stimulate macrophages to ingest and clear neutrophils (Serhan & Savill 2005).

In addition, neutrophils are prone to undergo apoptosis within hours after entering the inflammatory site. Thus, for on-going inflammation, continuous signaling for recruiting new neutrophils is essential. Such neutrophilic sensitivity to apoptosis can be dampened and postponed by inflammatory mediators that activate pro-survival and pro-inflammatory transcription factors such as NF-κB and Foxo3a (Serhan & Savill 2005). In turn, macrophages that are responsible for phagocytosing the apoptotic neutrophils can release pro-apoptotic signals such as Fas ligand, which can trigger apoptosis in the neighboring neutrophils (Henson 2005).

Macrophages engaged in uptake of apoptotic cells release inflammation-suppressing mediators such as transforming growth factor β1 (TGF-β1), which suppresses pro-inflammatory TLR signaling and participates in the resolution and healing/fibrosis phase, and vascular endothelial growth factor (VEGF), critical for repair of endothelial and epithelial injury. Thus, once apoptosis of leukocytes at the site of inflammation is initiated, the uptake of apoptotic cells causes a switch in the macrophage phenotype from activated and injurious to reparative and fibrotic (Nathan 2002, Henson 2005, Serhan & Savill 2005).

Systemic inflammation

The initial, appropriate inflammatory response to infection or injury may become amplified and dysregulated, resulting in a harmful or damaging host response. Systemic inflammation involves all cell and protein systems in the blood and affects inflammatory networks outside the circulation in secondary organs. The clinical syndrome is called the systemic inflammatory response syndrome. The clinical signs vary and in adults often include fever, confusion, transient hypotension, diminished urine output, and thrombocytopenia. An untreated systemic inflammatory response may progress to respiratory or renal failure, disseminated intravascular coagulation, and finally profound and unresponsive hypotension leading to death. The classical etiologic factor is septic infection, but massive trauma, burn injury, pancreatitis, and other states of significant tissue injury may lead to similar systemic activation of inflammation. The biological mechanisms resulting in these symptoms are massive release of vasoactive cytokines, lipid mediators and oxygen radicals as well as activation of coagulation parallel with activation of inflammation. The result is increased vasodilatation, fluid extravasation leading to hypotension, and enhanced coagulation leading to formation of microtrombi in small blood vessels, inadequate tissue perfusion, and organ failure (Cohen 2002, Russell 2006).

Inflammation is controlled by counter-regulatory mechanisms. These include soluble antagonists and receptors of pro-inflammatory cytokines blocking their action in tissues, inactivators of the complement and coagulation cascade, and anti-inflammatory cytokines such as interleukin-10 (IL-10) and TGF-β. In addition, the inflammatory cells are suppressed, reflected in increased lymphocyte apoptosis, T cell hyporesponsiveness and anergy, and myelomonocytic-cell deactivation leading to immunosuppression and inadequate host defence (Cohen 2002, Le Tulzo et al. 2002, Russell 2006). The clinical syndrome of lymphopenia, hypothermia, and susceptibility to nosocomial infection is referred to as the compensatory anti-inflammatory response syndrome (Cohen 2002, Han & Ulevitch 2005, Le Tulzo et al. 2002).

SPECIAL ASPECTS OF THE NEONATAL IMMUNE SYSTEM

Neonates, especially those born preterm, are considered immunocompromised, or as having their responses skewed. Clinically, this is evidenced by their increased risk for opportunistic infections such as bloodstream infections caused by commensal skin bacteria or candida species.

Breakdown of natural barriers

The skin of a newborn infant is more permeable than that of an adult. The normal bacterial flora of the skin and intestine is unestablished, which increases the risk for proliferation of pathogenic strains. However, the vernix caseosa lining the skin of newborn infants contains antibacterial substances aiding in protection before the establishment of the protective normal flora (Tollin et al. 2006, Levy 2007).

In preterm infants, the skin is even thinner and more permeable than in term infants, and its natural barrier function is further compromised by peripheral venous lines, heel prick sampling, arterial catheters, and central venous lines. Furthermore, intubation and mechanical ventilation provide a route for pulmonary infection, and the gastrointestinal tract is vulnerable due to nasogastric tubes and protein pump inhibitors sometimes used to prevent or treat gastric ulcers. The extensive use of antimicrobials before and after the birth postpones and skews the formation of natural microbial flora, further compromising the natural defence mechanisms of the skin and gastrointestinal tract.

Quantitative differences

Newborns have lower concentrations of many proteins of the innate immunity. The components of the complement are only 10 to 70% of adult values and correlate with gestational age (Levy 2007). In addition, concentrations of many acute-phase proteins such as mannose-binding lectin, soluble CD14, C-reactive protein, and LPS-binding protein are low at birth, but rise within the first week of life (Levy 2007). In the smallest preterm infants also the passive immunity provided by the maternal IgG antibodies is impaired or missing because the antibodies are transferred through the placenta to the fetus during the last two to three months of gestation.

Qualitative differences

Many qualitative differences in neonatal immunity can be explained by the newborn infant's lack of pre-existing immunological memory and differences in immune regulation. In addition, some functional differences in the innate immunity are present in newborns.

As compared to the phagocytes of an adult, neonatal phagocytes are less efficient in chemotaxis, rolling, adhesion, transmigration, and intracellular and extracellular bacterial

killing (Kenzel & Henneke 2006, Levy 2007). Neonatal neutrophils (Andersson et al. 1981, Bektas et al. 1990, Santos & Davidson 1993) and monocytes (Fanaroff & Martin 2000) accumulate less efficiently at sites of infection than in adults. Neonatal granulocytes migrate at reduced speed, and L-selectin-mediated rolling is lower (Levy 2007). In term infants, the phagocytic and microbicidal activity of phagocytes appears to be normal (Speer et al. 1986, 1988, Conly & Speer 1991). As compared with adults and healthy term infants, in preterm and septic or stressed term infants the neutrophil phagocytosis, respiratory burst activity and killing capacity are depressed (Gahr et al. 1985, Bortolussi et al. 1993, Falconer et al. 1995, Drossou et al. 1997). Furthermore, phagocytes' less efficient ability to release antimicrobial proteins in all newborns (lactoferrin, bactericidal/permeability-increasing protein) and in preterm infants (elastase) further contribute to their lower microbial killing capacity (Nupponen et al. 2002a, Gahr et al. 1987, Levy et al. 1999, Henneke et al. 2003).

In newborn infants, the cytotoxic T cell reactions are limited in magnitude, and the adaptive immunity is skewed towards Th2-type reactions (Adkins et al. 2004). Regulatory T cells that possess immunosuppressive functions are present in newborn peripheral blood, abundantly in preterm infants (Takahata et al. 2004).

Neonatal antibody responses are delayed in onset, reach lower peak levels, are of shorter duration, differ in distribution of IgG isotypes, and are of lower average affinity and reduced heterogeneity. This may be in part caused by the presence of maternal antibodies, as passive immunity prevents a full B cell response. There may be differences in neonatal B cells themselves, but this inefficiency is most probably due to immature Th cells co-stimulating the B cells (Adkins et al. 2004).

However, in many respects the neonatal adaptive immune system is similar to that of adults, and upon vigorous stimulation is capable of mature and full immune responses (Ridge et al. 1996, Sarzotti et al. 1996, Forsthuber et al. 1996, Adkins et al. 2004). The neonatal immune system must be plastic and flexible. After birth the immune system encounters environmental antigens as well as new self-antigens towards which it must establish tolerance but also encounters many potential pathogens threatening the host's survival. Thus, the neonatal immune system is characterized by a tendency to low responsiveness under non-threatening conditions, but is, however, capable of mature responses when the level of danger is high (Adkins et al. 2004).

PRENATAL INFLAMMATION

Intrauterine infection

Around 30 to 35% of preterm births are medically induced due to maternal or fetal problems, 40 to 45% are caused by spontaneous preterm labor, and 25 to 30% occur due to preterm prelabor rupture of the membranes (PPROM) (Goldenberg et al. 2008). Most spontaneous preterm births and PPROMs are caused by clinical or silent bacterial infection.

Chorioamnionitis is a polymicrobial bacterial infection of the placental membranes and amniotic fluid. It most often results from ascending infection by maternal vaginal bacteria, but can occur also by hematogenous spread. The infection promotes an intrauterine inflammatory reaction in the mother, reflected as increased pro-inflammatory cytokine concentrations such as IL-1, IL-6, and TNF-α in the amniotic fluid, which can initiate preterm labor (Gaudet & Smith 2001).

The maternal infection may spread to the fetus, as it is bathed in the amniotic fluid colonized by bacteria and rich in pro-inflammatory cytokines and receives its blood via the inflamed and infected placenta. In fact, in most cases of histologic chorioamnionitis, inflammation of the umbilical cord, funisitis, occurs as well, reflecting induction of fetal inflammatory response to the infection (D'Alquen et al. 2005). In addition, fetus-derived inflammatory cells can exist in the amniotic fluid and placenta during chorioamnionitis (Gomez et al. 1998).

Epidemiological data show that exposure to intrauterine inflammation reduces the incidence of RDS but raises the risk for chronic complications such as BPD and cerebral palsy (Watterberg et al. 1996, Kallapur & Jobe 2005, Gaudet & Smith 2001). It is assumed that inflammation may disturb organ development. Inflammatory cytokines such as IL-6 are present in amniotic fluid and are swallowed and aspirated by the fetus. The fetal lung maturation is accelerated with halting of the respiratory branching and initiation of septation and surfactant production. This results in functionally more mature lungs with smaller volumes (Levy 2007) and eventually aberrations in lung development characteristic of BPD (described in more detail below) (Thomas et al. 2008, Kramer 2008). At the same time, the white matter of the central nervous system is being myelinated, and neurons are migrating through the white matter to the cortical regions (Leviton & Gressens 2007). These processes may be vulnerable to damage by inflammatory substances, cytokines, and inflammatory cells.

Inflammation in pre-eclampsia

Pre-eclampsia is a maternal syndrome of unknown origin occurring after midgestation. Diagnostic criteria are an increase in maternal blood pressure over 140/90 mmHg and new-onset proteinuria of over 300 mg/24 hours (Roberts et al. 2003). Pre-eclampsia can progress to a convulsive phase, eclampsia, which is life-threatening to both the mother and fetus (Roberts et al. 2003). The only cure is removal of the placenta by birth. Thus, maternal pre-eclampsia often leads to medically induced birth or cesarean section before

completion of full gestation and is thus an important cause of preterm birth and associated problems.

In pathogenesis of pre-eclampsia, the key component is reduced placental perfusion, due to a primary abnormality in implantation and vascular remodeling (Roberts et al. 2003, Borzychowski et al. 2006). The resulting hypoxia in the fetal-maternal interface may result in generation of free oxygen radicals leading to oxidative stress in the blood and tissues of pre-eclamptic women (Roberts et al. 2003, Borzychowski et al. 2006). In addition, in the mother, systemic endothelial activation and activation of inflammation and coagulation occur (Borzychowski et al. 2006). In comparison to non-pregnant women, all pregnant women show endothelial activation and activation of coagulation, and pre-eclampsia occurs when one or several maternal systems decompensate (Redman & Sargent 2003, 2005, Borzychowski et al. 2006). The clinical syndrome of pre-eclampsia arises from circulatory disturbances caused by systemic maternal endothelial activation and dysfunction, which activates leukocytes and vice versa (Redman & Sargent 2005, Borzychowski et al. 2006).

In severe, particularly early-onset (prior to completion of 34 gestational weeks) pre-eclampsia, as a result of reduced blood flow in the placenta, the fetus often suffers from increasing nutritional and respiratory insufficiency which can lead to impaired growth, asphyxia, and even death (Redman & Sargent 2005).

POSTNATAL INFLAMMATION

Respiratory distress syndrome

The fetal lungs develop throughout the pregnancy, and alveolar development continues one to three years postnatally (see Figure 4 for schematic overview of lung development). At the end of the second trimester of pregnancy, the first gas exchanging areas are formed, and surfactant production begins, enabling survival outside the uterus. However, usually before 32 weeks' gestation the lungs are structurally and functionally immature, and birth before this time often results in progressive respiratory distress due to lack of surfactant, immature fluid transport, and lack of respiratory alveoli. The resulting clinical respiratory distress syndrome (RDS), occurring within hours after birth, is characterized by tachypnea, grunting, thoracic retractions, and need for supplemental oxygen.

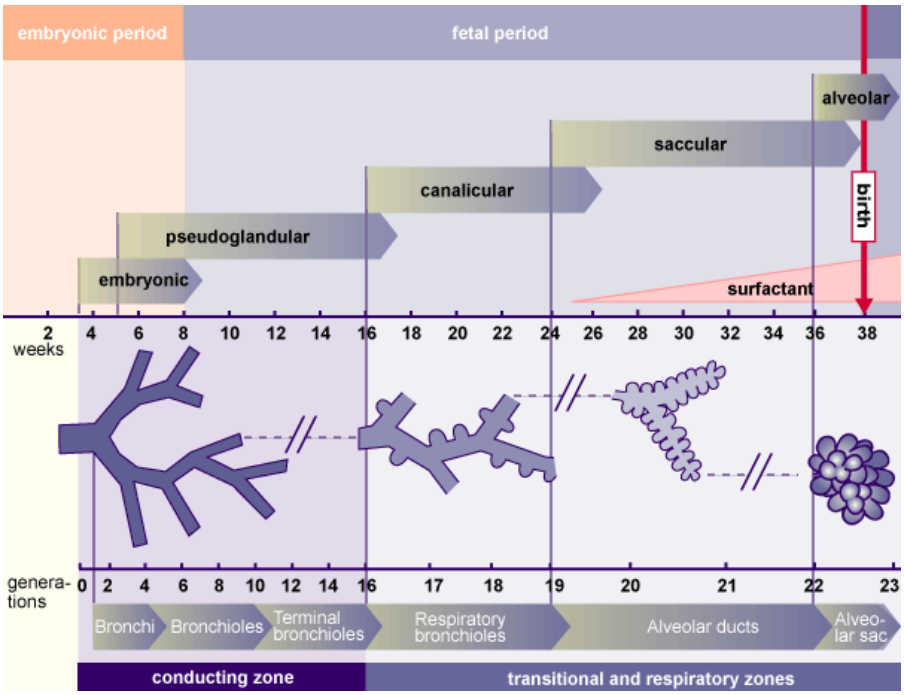

Figure 4 Overview of prenatal lung development. Note the timing of surfactant production and beginning of alveolarization. Reprinted with permission from copyright holder, *www.embryology.ch*.

Before the widespread use of assisted ventilation in the 1970's, the survival of preterm infants depended greatly upon the developmental stage of the lungs and especially their surfactant production. Consequently, very few survived before 28 weeks' gestation, and many even more mature infants died from respiratory distress (Saigal et al. 2008). Improved treatment strategies, including antenatal corticosteroid administration to the mother at risk for preterm delivery in order to accelerate fetal lung development, assisted ventilation and gentle ventilatory strategies, and exogenous surfactant administration improved survival rates of very preterm infants strikingly by the mid 1990's (Saigal et al. 2008, Engle et al. 2008).

Although assisted ventilation of preterm infants with RDS is crucial for survival, it also injures the immature lung. The mechanical stretching, high local pressures needed to keep the atelectasis-prone surfactant-deficient lung open, and high concentrations of oxygen required for adequate tissue oxygenation all contribute to this injury (Jobe et al. 1998, Kallapur & Jobe 2006). Proinflammatory cytokines are released by damaged endothelium and pneumocytes, and as a result, local inflammatory cells become activated and start recruiting circulating cells to the lungs (Tsuchida et al. 2006). Accumulation of neutrophils and monocytes to the lung parenchyma of preterm infants with RDS is well-documented (Merritt et al. 1981a, 1981b). Preterm infants with RDS show increased concentrations of proinflammatory cytokines in the lungs (Speer et al. 1993) and in the peripheral blood, together with systemic activation of leukocytes and the clotting cascade indicative of pulmonary and systemic inflammation (Murch et al. 1996a, 1996b, Nupponen et al. 2002b, Brus et al. 1994, 1997, Jaarsma et al. 2001).

Currently, RDS is treated immediately after delivery with administration of exogenous surfactant prophylactically to the infants at greatest risk for RDS, or later for established RDS diagnosed by an increasing oxygen requirement and characteristic radiological findings in the radiograph of the lungs (Engle et al. 2008). The use of more gentle ventilatory strategies and nasal continuous positive airway pressure (nCPAP) is now being recommended to prevent ventilator-induced lung injury (Ramanathan & Sardesai 2008, Rich et al. 2003).

Postnatal infections

Clinically, systemic infections in preterm infants are divided into two categories: early-onset and late-onset infections, with distinct etiologies and outcomes.

Early-onset infection presents during the first 72 hours after birth. The infection is acquired before or during the birth usually from the mother's genitourinary tract. Its incidence in very low birth weight (VLBW) (<1500 g) infants is in the US 15/1000 live births (McGuire et al. 2004). The main pathogens are Group B streptococci and *Eschericia coli*. Early-onset infection in VLBW infants increases mortality by three-fold to 40% as compared to infants of the same gestational age without infection (McGuire et al. 2004).

Late-onset infections are usually hospital acquired and become clinically evident more than 72 hours after birth, usually after the first week of life. The incidence of late-onset sepsis correlates inversely with birth weight. In extremely low birth weight (ELBW) (<1000 g) infants, the incidence of late-onset sepsis is 16 to 21%. In late-onset bloodstream infections in preterm infants, the most common pathogens are Gram-

positive bacteria (53 to 70%) followed by Gram-negative bacteria (18 to 22%) and fungal organisms (12 to 15%). Mortality is higher from Gram-negative bacteria (36%) and fungal organisms (32%) than from infections by Gram-positive bacteria (11%) (Fanaroff et al. 1998, Bizzarro et al. 2005, Stoll et al. 2002, 2004).

The initial clinical signs of sepsis in preterm infants are nonspecific and often subtle. However, the clinical course of the disease can be fulminant, and most deaths associated with late-onset infections in ELBW infants occur during the first few days (Stoll et al. 2002). Routine laboratory markers such as CRP or the immature/total neutrophil ratio are of limited value in aiding with diagnosis (Benitz et al. 1998, Ronnestadt et al. 1999, Krediet et al. 1992). Many soluble and cellular markers of inflammation have been evaluated, but no single marker is sufficiently sensitive and specific for neonatal sepsis (Ng & Lam 2006, Arnon & Litmanovitz 2008). Currently, antimicrobial therapy is started mainly on the basis of clinical signs. However, this strategy is problematic since it renders many infants unduly susceptible to side-effects of the drugs, disturbs the development of the normal microbial flora, and elevates risk for microbial antibiotic resistance.

Necrotizing enterocolitis

Necrotizing enterocolitis (NEC) is a neonatal bowel disease the incidence of which increases with decreasing gestational age. In addition to immaturity, enteral feedings, hypoxic-ischemic injury, and abnormal bacterial flora all play a role in the development of this disease (Lin & Stoll 2006). Its incidence among VLBW infants is approximately 1 to 10% with considerable variation between different centers, nations, and peoples (Lin & Stoll 2006).

The pathogenesis of NEC is incompletely understood, but the current concept is that both the intestinal epithelial-barrier functions and the innate immune responses to commensal bacteria are immature. As a result, intestinal innate immunity may react over-exuberantly to local microbes, resulting in disintegration of the gut epithelium (Lin & Stoll 2006, Hunter et al. 2008, Levy 2007). This results in bacterial invasion of the intestinal wall, which, in turn, initiates an inflammatory reaction and further damage. In the most severe form the whole thickness of the intestinal wall is destroyed, resulting in intestinal perforation, peritonitis, systemic bacterial invasion and sepsis. This devastating form of the disease requires surgery and is associated with a significant mortality of 50 to 70% (Lin & Stoll 2006, Hunter et al. 2008). The survivors suffer from several complications such as intestinal obstruction due to scarring, prolonged parenteral nutrition-associated liver failure, short-bowel syndrome, nutritional defects, and impaired growth and development (Martin et al. 2006, Hunter et al. 2008).

LONG-TERM COMPLICATIONS ASSOCIATED WITH INFLAMMATION

Bronchopulmonary dysplasia

The occurrence of respiratory problems in the preterm infant is biphasic. After clearance of RDS, or as a continuum of respiratory distress, or even without significant initial respiratory problems, the infant can develop a persistent dependence on supplemental oxygen and assisted ventilation. First described in the 1960s (Northway et al. 1967), this condition begun to emerge together with implementation of mechanical ventilation in preterm infants for treatment of RDS; the disease was designated bronchopulmonary dysplasia, or BPD. In that era, infants suffering from BPD had severe airway injury and fibrotic changes in the lungs which resulted in persistent respiratory dysfunction. Improved neonatal care and antenatal steroid treatment have lowered the threshold for survival of preterm infants, and the histopathology and characteristics of BPD have changed. Currently, BPD is occurring in more immature infants and is characterized by uniform arrest in lung development, with fewer and larger alveoli, reflecting inadequate septation, aberrant pulmonary microvascular development, but little or no airway injury or fibrosis (Jobe & Bancalari 2001, Kallapur & Jobe 2005, Kramer 2008). The so-called old BPD reflected injury to the rather well-developed lung, but now the new BPD is considered to result from aberrant development. In this thesis the term “BPD” refers to new BPD.

The current diagnostic criterion for BPD in infants born before completion of 32 gestational weeks is their need for supplemental oxygen at a post-menstrual age of 36 weeks. BPD is classified as moderate if oxygen requirement is less than 30%, and severe if it equals more than 30% (Jobe & Bancalari 2001).

Epidemiologic risk factors for BPD are exposure to chorioamnionitis, postnatal pulmonary and systemic infections, traumatic resuscitation, high inspiratory oxygen concentrations, and mechanical ventilation (Marshall et al. 1999, Speer 2006, Watterberg et al. 1996, Jobe & Bancalari 2001). A common antecedent behind these risk factors is inflammation (Kallapur & Jobe 2005). In addition, lack of growth factors such as VEGF inadequate nutrition, excess fluid intake, and patent ductus arteriosus may play a role in the development of BPD (Marshall et al. 1999, Lassus et al. 1999, Bose et al. 2008). See Figure 5 for an overview of the pathogenic mechanisms leading to BPD development.

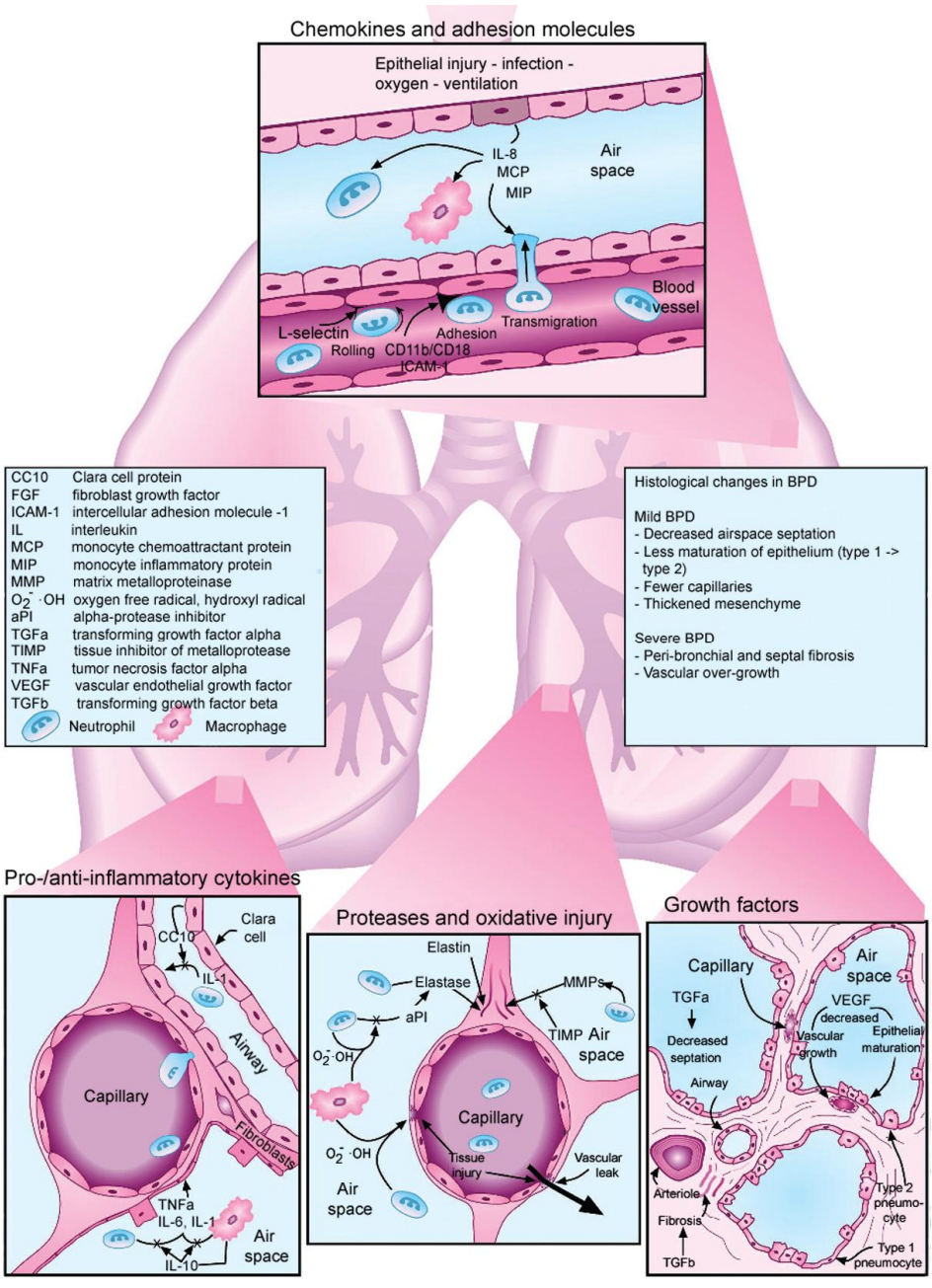
Chemokines and adhesion molecules
Epithelial injury - infection - oxygen - ventilation
IL-8
MCP
MIP
Air space
Blood vessel
Transmigration
L-selectin
Rolling
Adhesion
CD11b/CD18
ICAM-1
CC10 Clara cell protein
FGF fibroblast growth factor
ICAM-1 intercellular adhesion molecule -1
IL interleukin
MCP monocyte chemoattractant protein
MIP monocyte inflammatory protein
MMP matrix metalloproteinase
$O_2^- \cdot OH$ oxygen free radical, hydroxyl radical
aPI alpha-protease inhibitor
TGFa transforming growth factor alpha
TIMP tissue inhibitor of metalloprotease
TNFa tumor necrosis factor alpha
VEGF vascular endothelial growth factor
TGFb transforming growth factor beta
Neutrophil
Macrophage
Histological changes in BPD
Mild BPD
- Decreased airspace septation
- Less maturation of epithelium (type 1 -> type 2)
- Fewer capillaries
- Thickened mesenchyme
Severe BPD
- Peri-bronchial and septal fibrosis
- Vascular over-growth
Pro-/anti-inflammatory cytokines
Clara cell
CC10
IL-1
Airway
Capillary
Fibroblasts
TNFa
IL-6, IL-1
IL-10
Air space
Proteases and oxidative injury
Elastin
Elastase
aPI
MMPs
TIMP
Air space
$O_2^- \cdot OH$
Capillary
Tissue injury
Vascular leak
Growth factors
Capillary
Air space
TGFa
VEGF
decreased Vascular growth
Epithelial maturation
Decreased septation
Airway
Arteriole
Fibrosis
TGFb
Type 2 pneumo-cyte
Type 1 pneumocyte

Figure 5 The critical steps and associated mediators in lung inflammation, injury, and remodeling that result in bronchopulmonary dysplasia (BPD). Lung inflammation due to pre- or postnatal infection or lung injury, or both, is an important factor in the pathogenesis of BPD. Inflammation results in accumulation of inflammatory cells in the lung tissue as well as increased secretion of pro- and anti-inflammatory cytokines. Activated phagocytes release proteases contributing to the lung injury. In addition, the balance between local proteases such as matrix metalloproteinases and elastase and their inhibitors is disturbed, resulting in both lung injury and disturbance of normal lung development. The neutrophil activation and high concentrations of inspired oxygen lead to formation of reactive oxygen species. These are capable of causing tissue damage by lipid peroxidation in the basement membrane and other elements of the lung matrix, resulting in increased microvascular permeability and edema formation. Lung injury results also in abnormal production of growth factors leading to typical histological changes in BPD: decreased septation and alveolarization, aberrant microvascular development, and fibrosis. Modified from Bose et al. 2008 and reprinted with permission from the copyright holder, *Archives of Diseases of Childhood, Fetal and Neonatal Edition*.

BPD elevates the risk for neurologic sequelae such as cerebral palsy, neurosensory and motor abnormalities, and poor cognitive outcomes during childhood (Palta et al. 2000, Kobaly et al. 2008). It is associated with long-term impaired respiratory function and increased risk for life-threatening respiratory infections in childhood (Doyle et al. 2006, Kilbride et al. 2003). The chronic health problems associated with BPD have a major long-term influence on the everyday life of the families (Korhonen et al. 1999).

Since the pathogenesis of BPD is multifactorial (Figure 5), and no single treatment has proven successful in preventing or curing the disease, the current strategy for reducing BPD rates is complex. First, antenatal steroids should be given to mothers at risk for preterm birth to reduce the incidence and severity of RDS (Crowley 2008, Roberts & Dalziel 2008). For the infants at greatest risk for RDS, surfactant should be given prophylactically immediately after birth (Egberts et al. 1997, Soll & Morley 2008). Mechanical ventilation should be minimized, with nasal continuous positive airway pressure (CPAP) preferred whenever possible since it is less damaging to preterm lungs (Geary et al. 2008, Narendran et al. 2003, Meyer et al. 2004, Jegatheesan et al. 2006). Hyperoxia should be avoided by acceptance of lower tissue oxygen-saturation goals (Saugstad 2007). To optimize adequate nutrition, preterm infants should receive sufficient amino acid supplementation early (Geary et al. 2008). Care should be taken in prevention of nosocomial infections, and postnatal infections should be diagnosed early and treated vigorously. The use of methylxanthines for apnea of prematurity has also been shown to lessen the incidence of BPD (Schmidt et al. 2007). Postnatal corticosteroids that reduce the incidence of BPD elevate the risk for neurodevelopmental problems, so they are not recommended for BPD prevention (Halliday et al. 2008a, 2008b, 2008c) except in very high-risk populations (Doyle et al. 2005).

Neurological disorders

Perhaps the most important factor influencing the long-term outcome of preterm infants is their neurological outcome. Prematurity is associated with many factors threatening the normal development of the brain. It is estimated that up to 15% of the extremely low birth weight (<1000 g) infants develop cerebral palsy, and approximately half develop cognitive and behavioral deficits (Stoll et al. 2004). Cerebral white matter damage in the preterm infant (focal abnormalities, white matter volume loss, thinning of the corpus callosum, diffuse high signal intensity) detected by ultrasound or magnetic resonance imaging correlates with development of chronic neurologic sequelae (Glass et al. 2008).

Perinatal infections are risk factors for brain injury in term infants (Nelson et al. 1998). In preterm infants the evidence is controversial, and inflammation may even provide protection against damage to the developing brain (Andrews et al. 2008, Reiman et al. 2008, Nelson et al. 2003, Grether et al. 2003). However, both exposure to chorioamnionitis, especially when combined with a fetal inflammatory response (Dammann & Leviton 1997, 2006, Gaudet & Smith 2001, Edwards & Tan 2006), and recurrent postnatal infections (Glass et al. 2008, Stoll et al. 2004), are independent risk factors for white matter injury and poor neurological outcome and impaired growth. In addition, BPD is associated with neurologic sequelae (Palta et al. 2000, Kobaly et al. 2008).

The preterm brain may be vulnerable to inflammatory damage (Ugwumandu 2006, Edwards & Tan 2006). The migration of cortical neurons makes them susceptible to injury and to apoptotic cell death. The blood vessels perfusing the white matter are poorly developed and fragile, resulting in increased risk for intracerebral hemorrhage and ischemia. At the same time, neuronal axons are myelinizing, and cortical neurons are migrating through this mine-field of white matter undergoing injury, rendering them vulnerable to disturbances, injury, and cell death (Ugwumandu 2006, Leviton & Gressens 2007). In preterm infants, the blood-brain barrier of cerebral vessels preventing passage of most circulating molecules through the endothelium to the brain tissue is not fully developed and is permeable to pro-inflammatory cytokines – potent mediators of inflammatory injury in the perinatal brain. Furthermore, inflammatory, hypoxic-ischemic and metabolic insults may act synergistically to worsen neuronal damage.

In short, the brain of the preterm infant is exposed to numerous potentially injurious perinatal events evoking systemic inflammation. Due to ongoing development, the resulting impaired functions may not be due only to injury of preformed structures but also to aberrant brain development (Hagberg & Mallard 2005). As currently we have no means of protecting the developing brain from these attacks, we should aim at reducing the pulmonary injury, circulatory instability, infectious attacks, and metabolic and thermodynamic instability in order to reduce the neuronal injury and risk for neurodevelopmental sequelae.

AIMS OF THE STUDY

The aim of this study was to investigate systemic inflammation in preterm infants receiving intensive care. The ultimate aim is to understand the pathogenesis of the acute injury related to the common diseases of prematurity and mechanisms leading to chronic complications.

The specific aims of this study were in very low birth weight infants to:

I. Evaluate the value of phagocyte CD11b/CD18 expression in the diagnosis of late-onset sepsis

II. Investigate the timing, magnitude, and modulating aspects of systemic phagocyte activation in the first hours after the birth in infants with RDS

III. Study whether T lymphocytes are activated in RDS

IV. Study the effect of preterm labor and exposure to pre-eclampsia on systemic inflammation during the first week of life

PATIENTS AND METHODS

PATIENTS

The study projects were approved by the Ethics Committee for Pediatrics, Adolescent Medicine, and Psychiatry of the Helsinki University Central Hospital. Informed written consent was obtained from the parents. All blood samples were taken together with clinical sampling from indwelling arterial lines whenever possible or by heel-pricking, together with clinically indicated samples.

The study patients were recruited from the Neonatal Intensive Care Unit of the Hospital for Children and Adolescents or the Department of Obstetrics, both at Helsinki University Central Hospital.

Patient recruitment and allocation

The 32 patients for Study I were recruited between November 2000 and April 2002 and followed with daily blood samples for up to 4 weeks after birth or discharge, whichever came first. These patients were assigned to three groups: the infection group comprising seven infants with clinical signs of infection and blood culture positive for microbial growth, surgery for NEC, or both; the possible infection group comprising six infants, four with two or more clinical signs of infection and a blood culture test negative for microbial growth and two with no blood sample taken for culture; the control group of seven was collected from among study patients with no signs of infection by selecting a postnatal age-matched control for each infant with infection (Table 1).

Study II patients were recruited between July 2000 and September 2004. The 38 preterm infants intubated after birth and receiving either prophylactic surfactant or rescue surfactant or both, because of RDS, were classified as ventilated infants. These were compared with 25 preterm infants with mild or no RDS, never intubated and receiving no surfactant. Any suspicion of maternal infection (PPROM with high CRP, or fever or abnormal vaginal discharge) ensured the infant to be excluded.

The 55 preterm infants for Study III were recruited between July 2002 and April 2004; 34 infants requiring mechanical ventilation and surfactant therapy were classified as having RDS. These were compared with 21 infants with no need for surfactant or for ventilatory support (See Table 2). Infants who received postnatal hydrocortisone were excluded from analysis at subsequent time-points. One of the infants with RDS required surgery for necrotizing enterocolitis at the age of 5 days and was excluded.

The 36 preterm infants in Study IV were recruited between January 2003 and November 2004. These infants were allocated to two groups according to maternal diagnosis: 11 infants were allocated to the pre-eclampsia group, comprising the 9 born to

mothers with pre-eclampsia, defined as new-onset hypertension (>140/90 mmHg) and proteinuria (≥300 mg/24 hours) (Roberts et al. 2003) and 2 infants small for gestational age (SGA) (birth weight < -2SD) (Pihkala et al. 1989) with maternal hypertension (>140/90 mmHg) without proteinuria. Into the preterm-labor group we recruited 25 preterm infants born by preterm labor. During the study period, one infant developed blood culture-positive infection and one developed NEC. The CD11b expression values on the day of diagnosis and on the two preceding days and all the following days were excluded from analysis.

	I			II		III		IV	
	Infection	Possible infection	Controls	Ventilated infants	Controls	RDS	No RDS	Pre-eclampsia	Preterm labour
N	7	6	7	38	25	34	21	11	25
GA / weeks	24.9 (1.0)	26.6 (2.2)	26.8 (1.6)	28.3 (2.2)	32.1 (1.2) *	27.1 (2.0)	32.6 (1.4) *	27.7 (1.6)	26.1 (1.0) *
BW / grams	757 (132)	732 (104)	853 (102)	1086 (353)	1787 (457) *	900 (216)	1697 (406) *	740 (165)	868 (137) *
Male sex	4 (57)	1 (17)	4 (57)	18 (47)	15 (60)	14 (41)	12 (57)	2 (22)	14 (66)
Antenatal BM				35 (92)	18 (72)	33 (97)	21 (100)	11(100)	25(100)
Pre-eclampsia	1 (14)	4 (67)	1 (14)	11 (29)	10 (40)	5 (15)	8 (38)		
PPROM	3 (43)	1 (17)	0 (0)	4 (11)	7 (28)	11 (32)	7 (33)	0 (0)	13 (52) *
Chorioamnionitis	4 (57)	0 (0)	1 (14)	0 (0)	0 (0)	12 (36)	5 (24)	0 (0)	15 (60) *
Cesarean section	1 (14)	5 (83)	4 (57)	36 (95)	13 (52) *	23 (68)	12 (57)	11 (100)	5 (20) *
SGA						9 (26)	6 (29)	10 (91)	0 (0)
Multiple gestation						12 (35)	10 (48)	0 (0)	2 (8)
RDS	6 (86)	6 (100)	6 (86)					10 (91)	11 (44) *
IVH				7 (18.4)	1 (4.0)	9 (26)	3 (14)	2 (18)	11 (44)
LOS	6 (86)	0 (0)	0 (0)			10 (29)	1 (5) *	3 (27)	12 (48)
NEC	3 (43)	0 (0)	0 (0)			3 (9)	0 (0)	0 (0)	5 (20)
BPD				14 (37)	0 (0) *	18 (53)	0 (0) *	8 (73)	21 (84)
Death	1(14)	0(0)	1(14)	2(5)	1(4)	1(3)	0(0)	1 (9)	2 (8)

Table 1. Clinical characteristics of patients and controls in studies I, II, III, and IV. Shown as numbers as mean (SD) or n(%).* Denotes statistically significant ($p<0.05$) differences between study groups. Abbreviations: RDS – respiratory distress syndrome, GA – gestational age, BW – birth weight, BM – betamethasone, PE – pre-eclampsia, PPROM – preterm premature rupture of membranes, CA – (clinical) chorioamnionitis, CS – Cesarean section, SGA – small for gestational age, LOS – late-onset sepsis, NEC – necrotizing enterocolitis, BPD – bronchopulmonary dysplasia, IVH – intraventricular hemorrhage.

METHODS

Blood samples

Blood samples from the study infants were taken immediately after birth from the umbilical cord (II) and during the first 24 hours of life, before surfactant administration whenever possible and 1 and 2 hours after the surfactant and at 12 to 24 hours of age (II); during the first week of life (III and IV); and after the first week of life daily for 4 weeks (See Table 2 for details). Umbilical cord blood samples (II) were taken immediately after delivery with a sterile syringe. From newborn infants, blood samples were taken from arterial cannula whenever possible or by heel-pricking. Acid-citrate-dextrose (ACD) served as the anticoagulant.

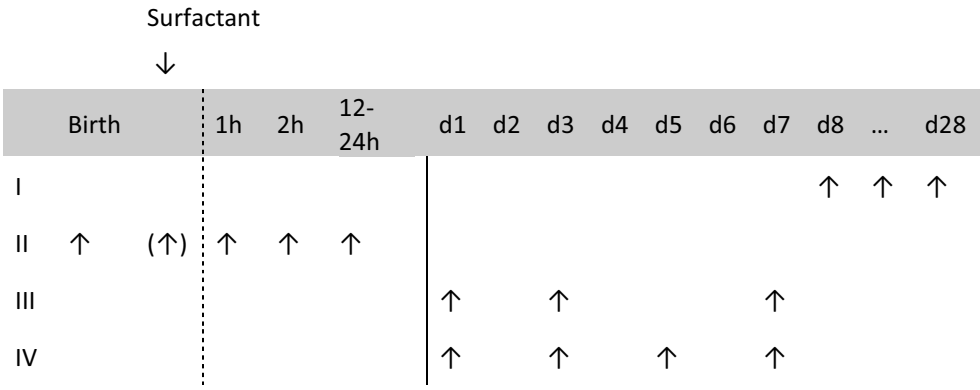

Table 2. Timing of blood sampling in Studies I, II, III, and IV. Birth →12-24h represents the first 24 hours of life, d1-d28 the following postnatal days.

Flow-cytometry

Sampling and sample preparation (I, II, III, and IV)

For flow-cytometric analysis of the phagocytes the samples were placed immediately after sampling in an ice-water bath to avoid *in vitro* up-regulation of phagocyte adhesion molecules. The samples for lymphocyte flow-cytometry were kept at room temperature for a maximum of 24 hours until analysis.

Phagocyte surface antigen expression analysis (I, II, and IV) was performed by two-color flow-cytometry. In brief, aliquots of whole blood (25 µl) were labeled within 24 hours from sampling with fluorescent anti-CD11b (phycoerythrin, PE) and anti-CD14 (fluorescein isothiocyanate, FITC) antibodies (BD Biosciences, San Jose, CA, USA) and incubated in the dark at 4°C. Red blood cells were lysed with FACS lysing solution (BENEX

Limited, BD Biosciences, Shannon, Country Clare, Ireland), and the cells were collected by centrifugation. The samples were resuspended in 1% formalin and kept at 4°C for up to 24 hours until analysis (Repo et al. 1993, 1995).

Lymphocyte subset and surface antigen expression analysis (III) was performed by two-color flow-cytometry. Aliquots of whole blood were stained at room temperature within 24 hours after sampling with monoclonal antibodies for CD4 or CD8, and CD54 or CD62L and LeukoGate (CD14-PE CD45-FITC; BD Biosciences). After the staining, the red blood cells were lysed with FACS lysing solution, the samples were washed, and the leukocytes were collected by centrifugation. The cells were resuspended in 1% formalin and kept at 4°C for a maximum of 24 hours until analysis.

Analysis of phagocytes (I, II, and IV)

For the flow-cytometric cell acquisition and analysis, a FACSort flow cytometer and CellQuest Pro analysis software (BD Biosciences) were used. Neutrophils were identified by their light-scatter pattern and monocytes by their light-scatter pattern and CD14 positivity. CD11b expression was reported in relative fluorescence units (RFU), i.e., the mean channel number of the positively fluorescent cells. A total of 2000 cells were collected for each analysis.

Analysis of lymphocytes (III)

From each sample, lymphocytes were first gated as CD14-negative and CD45-positive cells, and this gate served in the subsequent analysis. A total of 2000 lymphocytes were collected from each sample. Cells with fluorescence intensity higher than the fluorescence of cells stained with isotype controls were gated as positive. The percentages of CD4 and CD8 cells with increased expression of activation marker CD54 and those with decreased expression of CD62L were analyzed by flow cytometry and were divided with total CD4 and CD8 cell counts, respectively (i.e., CD4+CD54+/ CD4+tot).

White blood cell counts (III, IV)

The white blood cell counts (III and IV) and differentials (III) were performed in the clinical laboratory of the Hospital for Children and Adolescents, Helsinki, Finland (HUSLAB). A Sysmex XE-2100 (Sysmex, Kobe, Japan) hematology analyzer was used for cell differential counting. If the analyzer reported uncertain results, an experienced technician counted the differential with light microscopy.

Measurement of C-reactive protein (IV)

Concentrations of plasma CRP (I-IV) were measured with Modular immunoturbidimetry (Roche Diagnostics, Mannheim, Germany and Hitatchi Ltd, Tokyo, Japan) in HUSLAB.

Neurodevelopmental assessment (III)

The neurodevelopmental outcome of the infants with RDS in Study III was assessed at 2 years of corrected age by an independent pediatric neurologist and a neuropsychologist as a part of routine follow-up. Developmental assessment was conducted with a structured age-specific neurological examination (modified Touwen) (Touwen 1976) and a Griffiths Developmental Scale (DQ) (Brandt & Sticker 2001). Psychometric evaluation was based on the Mental developmental Index (MDI) of the Bayley Scales of Infant Development II (Bayley 1993). Severe neurodevelopmental abnormality was defined as cerebral palsy, blindness, deafness or severe developmental delay (MDI<70 or DQ<70, or both). Mild neurodevelopmental abnormality was defined as mild deviance in neurological examination (tone, posture, gross motor or fine motor function), strabismus, mild developmental delay (MDI /DQ >70 and < 85), or speech delay (vocabulary less than 20 words).

Statistical analysis

In Study, I the comparison of clinical data between the groups was done by analysis of variance (ANOVA), the Kruskall-Wallis test with exact significance, and the Fisher-Freeman-Halton test. Comparisons between two groups were performed with the Mann-Whitney U-test with exact significance. Changes during the days preceding infection were evaluated by the Page test for ordered alternatives with exact significance, and changes from Day -3 to Day 0 by Hodges-Lehmann estimate with 95% CI. Receiver-operating characteristics (ROC) curves served for determination of the threshold value for the infection group compared with the infants without infection, and the respective areas under the curve (AUC) with 95% CI were calculated with bias-corrected accelerated bootstrap (5000 replications).

In Study II, comparisons within groups between different time-points were carried out by Wilcoxon's Signed Rank test with 2-tailed exact significance, and comparisons between groups by the Mann-Whitney U-test with 2-tailed exact significance. Correlations were calculated by Spearman's correlation test with 2-tailed significance. The $\chi 2$ test was used to analyze categorial data, with Fisher's exact test when applicable.

In Study III, the skewed variables were log-transformed to attain normality. Two-way comparisons between subjects were made by Student's t-test and within subjects at two time points by paired t-test. Linear regression was used to adjust the data for potential confounding factors, with results expressed as unstandardized coefficients (B) with 95%

confidence intervals (CI). The χ2 test was used to analyze categorial data, with Fisher's exact test when applicable.

In Study IV, the skewed variables were log-transformed for normality, if possible. The arithmetic means of days 1 and 2, 3 and 4, and 5 and 6 (d1-2, d3-4, and d5-6) were used in data analysis to control for missing values. For normally distributed variables, Student's t-test was used; otherwise the differences were calculated by the Mann-Whitney U-test with exact significance. Categorical variables were analyzed with the χ^2 test. To adjust for potential confounding factors, linear regression was used, with the results expressed as unstandardized coefficients (B) with 95% confidence intervals (CI).

RESULTS

SYSTEMIC ACTIVATION OF PHAGOCYTES IN LATE-ONSET SEPSIS (I)

Patients (I)

In the infection group, one patient had a blood culture positive for *Staphylococcus epidermidis*, two for Gram-negative bacteria, and three for *Candida parapsilosis*. Three infants had surgery for NEC, and from two of them, blood samples were obtained for culture on the day of the bowel resection prior to surgery (Table 3).

Patient no	Days of age at day 0	Microbe in blood culture	Clinical data
1	11	*Proteus vulgaris*	NEC surgery at day 0
2	4	*Staphylococcus epidermidis*	NEC surgery at day 0
3	13	*Candida parapsilosis*	
4	8	*Serratia marcescens*	Death at day 0
5	12	*C. parapsilosis*	
6	17	*C. parapsilosis*	
7	6	(No blood culture)	NEC surgery at day 0

Table 3. Characteristics of infants with infection. Day 0 denotes for day of sampling for blood culture or surgery for NEC. Study I, reprinted with permission from the copyright holder, *Pediatric Research*.

Validation of the storage and sampling methods

To study the effect of 24-hour storage of the blood samples, we measured neutrophil and monocyte CD11b expression levels in a neonate without infection immediately after sampling and 24 hours later, taking some from the rest of the sample retained at 0°C. Coefficients of repeatability in samples from six infants were for neutrophils 27 RFU, and monocytes 19 RFU, indicating a 95% expectation that neutrophil values will differ by less than 27 RFU and monocyte values by less than 19 RFU between samples stored at 0°C up to 24 hours.

In addition, to validate different sampling methods, we measured CD11b expression on phagocytes of healthy adult volunteers from blood samples taken by venipuncture and from three consecutive drops of blood obtained by fingertip pricking, using the same system as in newborns. A significant agreement in CD11b expression existed between the sample obtained by venipuncture (median 150 RFU, range: 66-225, in neutrophils and 151 RFU, 74-183, in monocytes) and the three other samples obtained by fingertip

pricking (152 RFU, 75-223, in neutrophils; 142 RFU, 85-184, in monocytes). The intraclass correlation coefficients with 95% confidence intervals were for neutrophils 0.91 (95% CI: 0.84 to 0.97) and monocytes 0.82 (95% CI: 0.67 to 0.92).

CD11b expression in late-onset sepsis (I)

In the infection group, CD11b expression increased from 3 to 0 days prior to the day of blood culture in both neutrophils (p for trend; p=0.006) and monocytes (p<0.001) (Figure 6). At Day 0, median CD11b density was higher in the infection group than in controls on both neutrophils (p=0.001) and monocytes (p<0.001). Results for ROC analysis are presented in Table 4.

	Neutrophils (cut-off: 139 RFU)	Monocytes (cut-off: 225 RFU)
Sensitivity	1.00 (0.59-1.00)	0.86 (0.42-1.00)
Specificity	0.56 (0.30-0.80)	0.94 (0.70-1.00)
PPV	0.50 (0.22-0.77)	0.86 (0.42-1.00)
NPV	1.00 (0.66-1.00)	0.94 (0.70-1.00)
AUC	0.83 (0.57-0.96)	0.95 (0.72-1.00)

Table 4. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC) (95% confidence interval) in preterm infants for late-onset sepsis or necrotizing enterocolitis.

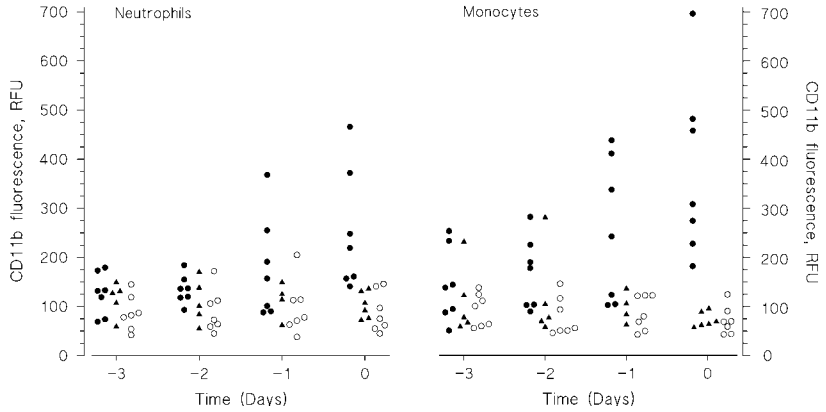

Figure 6 CD11b expression on neutrophils and monocytes in preterm infants with infection (•), possible infection (▲) and without infection (○) on Day 0 (day of sampling for blood culture or surgery for necrotizing enterocolitis) and 3 preceding days. Study I, reprinted with permission from the copyright holder, *Pediatric Research*.

SYSTEMIC ACTIVATION OF PHAGOCYTES IN RESPIRATORY DISTRESS SYNDROME (II)

Patients (II)

Of the 38 ventilated infants, 23 received only rescue surfactant at the age of 1 to 4 hours, and 15 received prophylactic surfactant in the delivery room within 15 min after birth, or received both. Rescue surfactant was given if the required FiO_2 exceeded 30%, or if a radiograph of the lungs showed changes typical for RDS. Of the controls, eight required respiratory support by means of nCPAP (5 cm H_2O) at from 1 to 12 hours of life. All the infants had negative blood culture results, and none had signs of early-onset infection. A chart of showing ventilatory treatment of the ventilated infants and controls is in Table 5.

Ventilated infants (38) SIMV (37) / HFV (1)		Controls (25)	
↓	↓	↓	↓
Prophylactic surfactant (15)	Rescue surfactant (23)	nCPAP (8)	No respiratory support (17)
↓			
Additional doses of surfactant (11)			

Table 5. Treatment of ventilated infants and controls during the first 12-24 hours of life. Abbreviations: SIMV – synchronized intermittent mandatory ventilation, HFV – high-frequency ventilation, nCPAP - nasal continuous positive airway pressure.

CD11b expression in RDS (II)

In the controls, neither neutrophil nor monocyte CD11b expression increased significantly from birth to the age of 2 to 6 h or between the ages of 2 to 6 h and 12 to 24 h. In addition, phagocyte CD11b expression was similar at all time-points in infants treated with nCPAP and in those without respiratory support (Table 6).

	CD11b expression / RFU		
	Cord blood	2-6 h of age	12-24 h of age
Infants with nCPAP (N=8)			
neutrophils	84 (77-99)	87 (71-161)	115 (75-198)
monocytes	77 (70-102)	80 (64-133)	97 (69-127)
Infants without nCPAP (N= 17)			
neutrophils	86 (66-102)	132 (98-291)	123 (81-150)
monocytes	69 (53-84)	90 (67-219)	175 (82-234)

Table 6. Expression of CD11b in control infants treated with nasal continuous positive airway pressure (nCPAP) and without respiratory support. Values are medians (quartiles). Reproduced with permission from *Pediatrics*, Vol. 117, Pages: 448-454, Copyright © 2006 by the AAP.

In ventilated infants, neutrophil and monocyte CD11b expression increased significantly from birth to samples taken 1 h and 2 h after surfactant; these increased further by the age of 12 to 24 h (Figure 7). In the 23 infants receiving only rescue surfactant, neutrophil CD11b expression was already significantly increased before the first dose of surfactant compared with birth values (p=0.001) (Table 7). In the subgroup of 15 infants receiving prophylactic surfactant, phagocyte CD11b expression increased from birth to 1 h and 2 h after surfactant, in a similar manner as in infants receiving only rescue surfactant (Table 7).

		CD11b expression / RFU				
		Cord blood	Before surfactant	1h after surfactant	2h after surfactant	12-24h of age
Prophylactic surfactant (n=15)	Neu	88 (68-105)		131 (85-334)	108 (68-151)	202 (154-325)
	Mon	94 (88-115)		72 (56-233)	80 (67-99)	233 (146-352)
Rescue surfactant (n=23)	Neu	78 (65-86)	107 (79-197)	98 (86-137)	123 (96-165)	157 (118-190)
	Mon	88 (70-102)	91 (70-121)	105 (82-138)	115 (90-165)	151 (117-221)

Table 7. Expression of CD11b in ventilated infants receiving prophylactic surfactant and rescue surfactant. Values are median (quartiles). Neu – neutrophils, Mon – monocytes. Reproduced with permission from *Pediatrics*, Vol. 117, Pages: 448-454, Copyright © 2006 by the AAP.

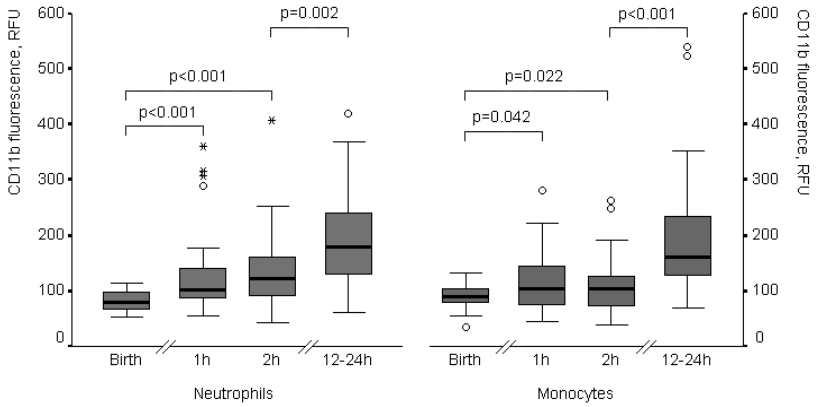

Figure 7 Expression of CD11b on circulating neutrophils and monocytes in ventilated preterm infants at birth and 1 and 2 hours after the first surfactant, and at 12 to 24 hours of age. Lines represent medians, boxes 50th percentiles and whiskers range. Circles (○) represent outliers with more than 1.5 times interquartile range outside median and asterisks (*) represent extreme outliers more than 3 times interquartile range from median. Reproduced with permission from *Pediatrics*, Vol. 117, Pages: 448-454, Copyright © 2006 by the AAP.

Compared with the controls, neutrophil and monocyte CD11b expression in ventilated infants was significantly higher at 24 h of age (Figure 8). No differences existed in CD11b expression between controls and ventilated infants in cord blood or at 2 h.

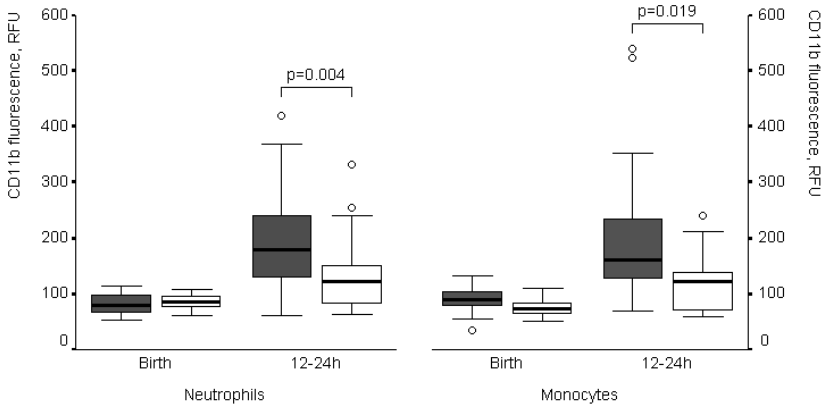

Figure 8 Expression of CD11b on circulating neutrophils and monocytes in ventilated preterm infants (gray boxes) and in preterm infants without mechanical ventilation (white boxes) at birth and at 12 to 24 hours of life. Reproduced with permission from *Pediatrics*, Vol. 117, Pages: 448-454, Copyright © 2006 by the AAP.

In ventilated infants, CD11b expression on neutrophils at 1 h after surfactant showed a positive correlation with gestational age ($r=0.548$, $p<0.001$), and expression on neutrophils and monocytes at 2 h after surfactant also showed positive correlations with gestational age (neutrophils: $r=0.584$, $p<0.001$; monocytes: $r=0.376$, $p=0.03$). CD11b expression at birth or at 12 to 24 hours of age did not correlate with gestational age. Indices of lung morbidity, i.e., arterial/alveolar PO_2 ratio, number of surfactant doses, or maximal FiO_2 during the first 24 hours did not correlate with phagocyte CD11b expression. In ventilated infants, phagocyte CD11b expression on the first day of life was comparable in infants who developed BPD to the expression in those who did not.

In the controls, CD11b expression on neutrophils or monocytes correlated with gestational age in none of the samples.

ACTIVATION OF CIRCULATING T LYMPHOCYTES IN RESPIRATORY DISTRESS SYNDROME (III)

Patients (III)

The clinical characteristics of the infants with and without RDS are given in Table 1. Infants with RDS were of lower gestational age and birth weight than were infants without RDS. BPD was diagnosed in 18 of the infants with RDS and in none of the infants without RDS. All infants who received postnatal hydrocortisone were excluded from the analysis at subsequent time-points. In all infants the initial blood cultures were negative. One of the infants with RDS required surgery for necrotizing enterocolitis at the age of 5 days and was excluded from analysis.

Absolute numbers of circulating CD4 and CD8 cells (III)

The number of CD4 cells increased from day 1 to day 7 ($p=0.004$), and from day 3 to day 7 ($p=0.004$) in infants with RDS, but remained stable in infants without RDS (Figure 10). On day 1 no difference appeared in number of CD4 cells between infants with RDS and without. On day 3, the number of CD4 cells was lower in infants with RDS ($p=0.02$). This difference remained statistically significant when adjusted for gestational age, maternal chorioamnionitis, mode of delivery, and time between antenatal betamethasone administration and birth ($p=0.02$). On day 3 a negative correlation occurred that did not reach statistical significance between gestational age at birth and number of CD4 cells in infants with RDS ($r= -0.404$, $p=0.08$). Gestational age and number of CD4 cells did not correlate in infants without RDS ($r=-0.389$, $p=0.2$). On day 7, no statistically significant differences existed between the groups.

The number of CD8 cells increased from day 1 to day 7 in infants with RDS ($p=0.03$, Figure 9), but remained unchanged in infants without RDS. In infants with RDS the number of CD8 cells on day 1 was lower than in infants without RDS at marginal statistical significance ($p=0.06$). On day 3, the number of CD8 cells in infants with RDS was lower ($p=0.02$). This difference remained statistically significant when adjusted for gestational age, maternal chorioamnionitis, mode of delivery, time between administration of antenatal betamethasone and birth, and being small for gestational age ($p=0.02$). On day 3, a significant negative correlation emerged between gestational age and number of CD8 cells in infants with RDS ($r= -0.45$, $p=0.04$). Gestational age and number of CD8 cells showed no correlation in infants without RDS ($r= -0.35$, $p=0.3$). Day 7 figures lacked any statistically significant differences between groups.

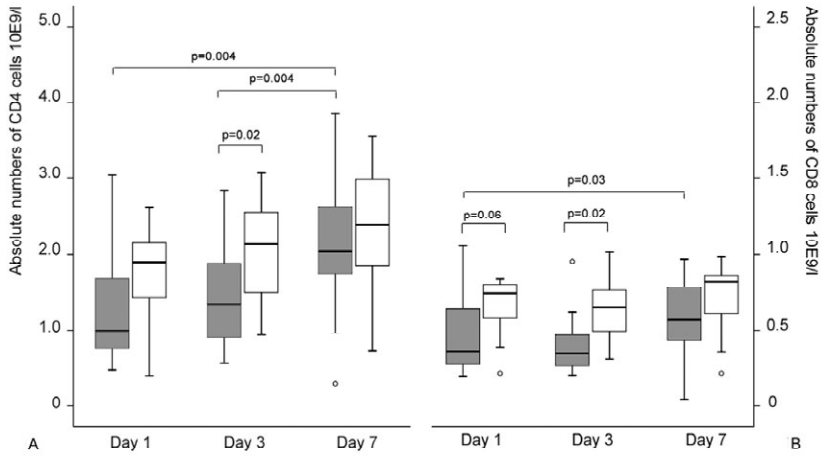

Figure 9 Absolute numbers of circulating CD4 cells and CD8 cells in infants with RDS (gray boxes) and infants without RDS (white boxes) on postnatal days 1, 3, and 7. Note different scales on y-axis in A and B. Adjusted p-values are given. Modified from Study III and reprinted with permission from the copyright holder, *Neonatology,* S. Karger AG, Basel.

T cell activation markers and RDS (III)

On day 1, the proportion of CD4 cells expressing CD54 (CD4+CD54+) was higher in infants with RDS than in infants without RDS (p=0.001) (Figure 10). When adjusted for mode of delivery, maternal chorioamnionitis, time between administration of antenatal betamethasone and birth, and being born SGA, the difference remained similar and statistically significant (p=0.001), but when gestational age was entered into the equation, this difference was no longer statistically significant (p=0.4). On day 3, the proportions of CD4+CD54+ cells were higher in infants with RDS, with marginal statistical significance (p=0.06). After adjustment for mode of delivery, maternal chorioamnionitis, time between antenatal betamethasone administration and birth, and being born SGA, the difference remained similar and was statistically significant (p=0.03). Again, entering gestational age into the equation resulted in non-significant results (p=0.9). Day 7 showed no statistically significant differences between groups. In infants with RDS, the proportion of CD4+CD54+ cells decreased from day 1 to day 7 with marginal statistical significance (p=0.06) (Figure 10). The proportions of CD8+CD54+ cells and CD4+CD62L+ cells were similar in infants with and without RDS on days 1, 3, and 7.

In infants with RDS, on day 1, the proportions of CD8+CD62L+ cells were lower than in infants without RDS (p=0.03, Figure 10). When adjusted for gestational age, mode of delivery, maternal chorioamnionitis, time between administration of antenatal betamethasone and birth, and being born SGA, the difference remained similar and was statistically significant (p=0.02). On day 3 in infants with RDS, the proportions of CD8+CD62L+ cells were lower than in infants without RDS (p=0.01, Figure 10). This difference remained statistically significant after adjustment for mode of delivery,

maternal chorioamnionitis, time between administration of antenatal betamethasone and birth, and being born SGA (p=0.02). However, entering gestational age into the equation resulted in a non-significant result (p=0.8). Day 7 revealed no statistically significant differences between the groups.

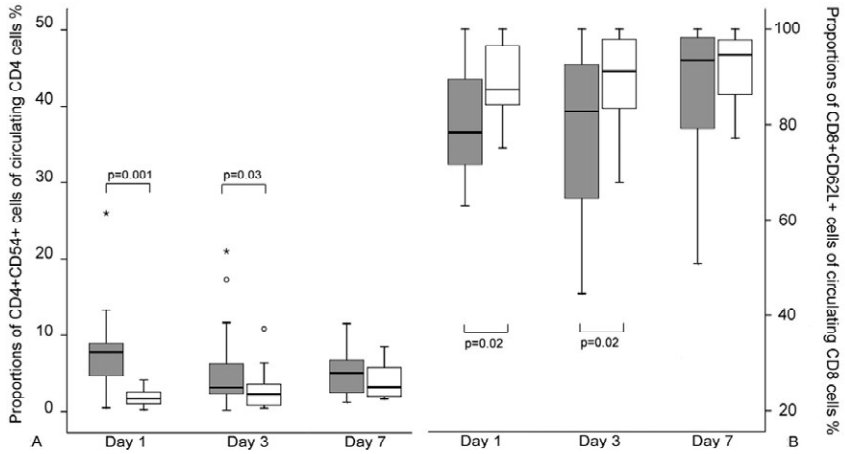

Figure 10 Proportions of CD4+CD54+ cells of circulating CD4 cells (A) and of CD8+CD62L+ cells of circulating CD8 cells (B) in infants with RDS (gray boxes) and without RDS (white boxes) on postnatal days 1, 3, and 7. Note different scales on the y-axis in A and B. p-values are adjusted for mode of delivery, maternal chorioamnionitis, time between administration of antenatal betamethasone and birth, and being born small for gestational age, but not for gestational age. Modified from Study III and reprinted with permission from the copyright holder, *Neonatology,* S. Karger AG, Basel.

T cell activation markers and BPD and neurodevelopmental outcome (III)

Of the infants with RDS, 18 developed BPD and 16 did not. The infants with BPD were significantly more immature and were of lower birth weight than infants without BPD (**III:** Table 2). They also more often had been exposed to chorioamnionitis (**III:** Table 2). The circulating numbers of CD4 and CD8 cells were similar in these infants (data not shown). Infants who developed BPD had higher proportions of CD4+CD54+ cells on day 3 (p=0.01) and CD8+CD54+ cells on day 1 (p=0.01) and day 3 (p=0.04) as compared with values in infants without BPD (**III:** Figure 3). No statistically significant differences appeared in proportions of CD4 and CD8 cells expressing CD62L between infants with and without BPD.

Of infants with RDS, the neurodevelopmental outcome was normal in 56%, mildly abnormal in 18%, and severely abnormal in 6% of the infants; in five infants (15%) no assessment was available. No correlations appeared between T cell counts or numbers of activated cells and neurodevelopmental outcomes.

INCREASED INFLAMMATION IN PRETERM INFANTS BORN TO MOTHERS WITH PRE-ECLAMPSIA (IV)

Patients (IV)

Infants born after maternal pre-eclampsia were of higher gestational age but lower birth weight than were infants born after preterm labor; 10 of 11 infants in the pre-eclampsia group were SGA. Rate of cesarean section was higher for infants born after pre-eclampsia. None of the pre-eclamptic mothers had chorioamnionitis or PPROM. Infants born after maternal pre-eclampsia more often (91%) received rescue surfactant after the prophylactic surfactant than did infants born after preterm labor (44%). No differences occurred in incidence of patent ductus arteriosus, late-onset sepsis, and necrotizing enterocolitis and rates of BPD or death between groups.

Infants born after the pre-eclampsia had lower total white blood cell counts throughout days 1 to 7 and higher CRP concentrations on days 2 to 6 as compared with infants born after preterm labor (**IV:** Table 2).

CD11b expression after pre-eclampsia and preterm labor (IV)

During the first week of life, the CD11b expression on circulating phagocytes was higher in infants born after pre-eclampsia than in infants born after preterm labor: neutrophils mean (SD), on days 1 to 2: 291(74) vs. 167(53) RFU, $p<0.001$; days 3 to 4: 269(63) vs. 191(61), $p=0.002$; and days 5 to 6: 200(62) vs. 156(43), $p=0.02$ (Figure 11); and monocytes on days 1 to 2: 323(137) vs. 155(65) $p=0.001$; days 3 to 4: 258(101) vs. 179(74), $p=0.02$; and days 5 to 6: 207(79) vs. 146(46), $p=0.007$ (Figure 12). On day 7, no statistically significant differences emerged between the infants: neutrophils 163(54) vs. 158(46), $p=0.8$; and monocytes 200(127) vs. 174(51), $p=0.5$. When adjusted for antenatal covariates (gestational age, sex, time from antenatal betamethasone to birth) the differences remained similar and statistically significant for neutrophils on days 1 to 2 ($p=0.02$) and 3 to 4 ($p<0.001$) (**IV:** Table 3), and for monocytes on days 1 to 2 ($p=0.04$) and 3 to 4 ($p=0.01$) (**IV:** Table 3). When adjusted for postnatal covariates (requirement of additional doses of surfactant and daily FiO2), the differences remained similar and statistically significant for neutrophils on days 1 to 2 ($p<0.001$) and 3 to 4 ($p=0.02$) (**IV:** Table 3), and for monocytes on days 1 to 2 ($p=0.005$), 3 to 4 ($p=0.02$), and 5 to 6 ($p=0.03$) (**IV:** Table 3).

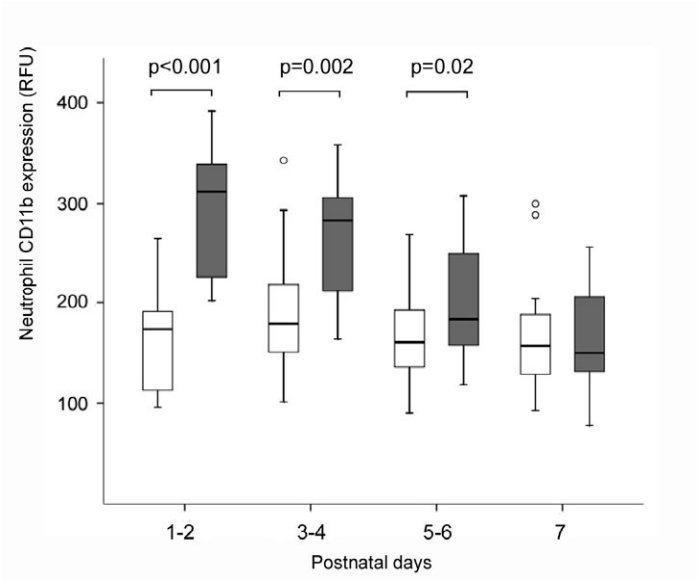

Figure 11 CD11b expression (RFU) on neutrophils during postnatal days 1-2, 3-4, 5-6 and 7 in preterm infants born after preterm labor (white boxes) and pre-eclampsia (gray boxes). Unadjusted p-values are given. For adjusted p-values see IV, Table 3. Modified from Study IV.

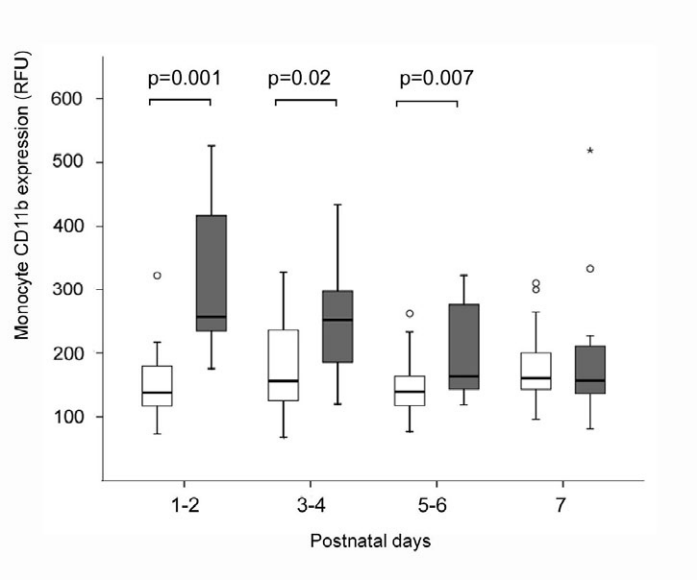

Figure 12 CD11b expression (RFU) on monocytes during postnatal days 1-2, 3-4, 5-6 and 7 in preterm infants born after preterm labor (white boxes) and pre-eclampsia (gray boxes). Unadjusted p-values are given. For adjusted p-values see IV, Table 3. Modified from IV.

Among infants born after preterm labor, no difference appeared in CD11b expression between 15 infants born to mothers with clinical chorioamnionitis as compared with those 10 born to mothers without chorioamnionitis: CD11b expression on neutrophils in infants exposed to chorioamnionitis vs. no chorioamnionitis, mean(SD) on days 1 to 2: 155 (58) vs. 184 (43), p=0.4; days 3 to 4: 195 (71) vs. 186 (46), p=0.7; and days 5 to 6: 146 (38) vs. 171 (48), p=0.2 and on monocytes on days 1 to 2: 139 (84) vs. 173 (33), p= 0.4; days 3 to 4: 205 (76) vs. 143 (55), p=0.06; and days 5 to 6: 145 (38) vs. 147 (58), p=0.9.

DISCUSSION

ANTENATAL FACTORS AND POSTNATAL INFLAMMATION (IV)

In this thesis we evaluated the effect on postnatal inflammation in preterm infants of two distinct antenatal factors: maternal pre-eclampsia and spontaneous preterm labor (Study IV). We found high systemic inflammation in preterm infants born to pre-eclamptic mothers, as demonstrated by higher phagocyte CD11b expression levels and CRP concentrations. Unexpectedly, lower postnatal inflammationin the preterm infants was associated with preterm labor. Antenatal factors are known to influence the preterm infant's postnatal morbidity. Maternal chorioamnionitis associates with inflammatory response in the fetus, (Gomez et al. 1998, Nupponen et al. 2000), and in the infant with reduced risk for RDS but increased risk for long-term conditions such as BPD, and at least in term infants with cerebral white matter damage, and with cerebral palsy (Watterberg et al. 1996, Gonzalez et al. 1996, Marshall et al. 1999, De Dooy et al. 2001, Speer 2006, Dammann et al. 2005, Edwards & Tan 2006, Nelson et al. 1998). Study II focused on postnatal inducers of inflammation and therefore excluded infants born to mothers with suspected chorioamnionitis or PPROM. Infants in Study II showed no signs of antenatal inflammation, and cord blood phagocyte CD11b expression was consistently low. In Study III we adjusted the results for maternal chorioamnionitis. The results for lymphocyte activation in RDS were not affected by maternal chorioamnionitis. As incidence of chorioamnionitis was significantly higher in infants later developing BPD (**III:** Table II), it is possible that the increased T cell activation in infants developing BPD may reflect exposure to chorioamnionitis, more severe RDS, or both.

Pre-eclampsia is known to be correlated with systemic activation of inflammation in the mother as demonstrated by high neutrophil CD11b expression and concentrations of CRP, as well as other mediators of inflammation such as lactoferrin and calprotectin (Tsukimori et al. 2008, Sabatier et al. 2000, Garcia et al. 2007, Üstün et al. 2005, Braekke et al. 2005). Increased superoxide production has been reported in *in vitro* stimulated neutrophils drawn from pre-eclamptic mothers (Tsukimori et al. 2008). Furthermore, culture medium from pre-eclamptic placental villous culture is capable of stimulating neutrophils *in vitro* (Wang et al. 2001). Pre-eclamptic mothers have lower plasma glutamine concentrations than do normotensive pregnant women (Hsu et al. 2005). Glutamine attenuates inflammation by downregulating expression of the circulating mediators of inflammation (Singleton et al. 2008). Whether the lower glutamine levels in the pre-eclamptic mothers lead to reduced glutamine concentration and thus to reduced anti-inflammatory capacity in the fetus remains to be investigated (Cetin et al. 2005, Battaglia & Regnault 2001). Interestingly, increased neutrophil CD11b expression has been found in cord blood from infants born after pre-eclampsia, suggesting intrauterine neutrophil activation (Saini et al. 2004).

LUNG INJURY AND SYSTEMIC INFLAMMATION (II AND III)

The main emphasis of this thesis is systemic inflammation associating with RDS. The findings of Studies II and III indicate that in preterm infants mechanically ventilated due to RDS, both circulating phagocytes and T lymphocytes are activated. Systemic phagocyte activation, evidenced by increased CD11b expression on circulating phagocytes, begins within hours after the start of mechanical ventilation. It is followed by systemic T cell activation during the first days of life. T cell activation is evidenced in peripheral blood by reduced T cell numbers and increased proportions of activated T cells.

Study II finds evidence that in preterm infants with RDS, initiation of mechanical ventilation, but not nCPAP, is associated with onset of systemic inflammation. Inflammation was detectable neither in cord blood samples nor in newborn preterm infants without ventilatory support. The inducer of inflammation may thus be mechanical ventilation. NCPAP treatment has, in an animal model, been shown to correlate with decreased indicators of lung injury (Jobe et al. 2002), and epidemiologically in human preterm infants to improve overall outcome when compared with mechanical ventilation (Polin & Sahni 2002, DeKlerk & DeKlerk 2001, Ramanathan & Sardesai 2008, Rich W et al. 2003, Geary et al. 2008, Narendran et al. 2003, Meyer et al. 2004, Jegatheesan et al. 2006). This may, at least in part, be due to less lung injury with nCPAP, resulting in a milder systemic inflammatory response.

As shown in animal studies, mechanical ventilation of the immature lung leads to rapid induction of pulmonary and systemic inflammation (Jaarsma et al. 2001 and 2004, Ikegami et al. 2000, Ikegami & Jobe 2002, Naik et al. 2001). In animal models, the preterm lung is even injured by a few manual ventilations at birth (Björklund et al. 1997), and proinflammatory mediators are expressed as early as two hours after onset of mechanical ventilation, despite supplemental surfactant (Naik et al. 2001). Furthermore, mechanical stretching of rat alveolar type II cells in vitro has resulted in increased release of pro-inflammatory and decreased release of anti-inflammatory cytokines (Hammerschmidt et al. 2005), which bear the potential to activate isolated rat lymphocytes to increase their CD54 expression (Hammerschmidt et al. 2005). Mechanical ventilation of the immature lung causes edema, hemorrhage, and epithelial necrosis, it compromises lung mechanics, inhibits surfactant function, and promotes expression of proinflammatory cytokines (Ikegami et al. 2000, Ikegami & Jobe 2002, Naik et al. 2001, Attar & Donn 2002). Distressed, injured, or dying cells release substances that tissue antigen-presenting cells sense as alarm signals (Matzinger 2002). Our findings and previous reports show that mediators of acute lung injury are capable of activating the innate as well as the adaptive immune system (Hammerschmidt et al. 2005, Ballaph et al. 2003).

Administration of surfactant to a preterm infant with RDS improves lung function and thus enables more gentle ventilation. In addition, the natural porcine surfactant (Curosurf®) used in these studies has been shown to have anti-inflammatory effects *in vitro* (Baur et al. 1998). On the other hand, natural porcine surfactant has also been shown to contain platelet-activating factor, a strong neutrophil activator (Moya et al. 1993), and surfactant may also have proinflammatory effects. However, based on our findings, administration of natural porcine surfactant exerts no significant influence on

phagocyte activation in mechanically ventilated preterm human infants. This is in harmony with findings in animal models demonstrating that even with surfactant replacement therapy and lung protective ventilatory strategies, mechanical ventilation initiates lung inflammation (Ikegami et al. 2000, Ikegami & Jobe 2002, Naik et al. 2001).

A relative lymphopenia occurred in preterm infants with RDS (III). Activated lymphocytes may leave the circulation and be recruited to the tissues or may enter the lymphatic system. Neutrophils and monocytes accumulate in the lungs of infants suffering from RDS. Data concerning lymphocytes in the lungs of preterm infants with RDS are scarce, but in a baboon model for BPD, CD4 cells in the lung interstitium are increased (Rosen et al. 2006). Whether the decreased number of peripheral T cells in infants with RDS is due to the recruitment of these cells to tissues remains to be elucidated. Another cause for a reduced number of peripheral lymphocytes could be increased apoptosis. Leukopenia is a common finding in infants with septic infections. Septic shock in adults may lead to a compensatory anti-inflammatory response syndrome with relative lymphopenia due to increased apoptosis (Le Tulzo et al. 2002). Ventilated surfactant-deficient rats present with peripheral immunosuppression (Vreugdenhil et al. 2006). If such an anti-inflammatory state is present also in mechanically ventilated preterm infants, it may play a part in their increased susceptibility to infections.

In the pathogenesis of BPD, inflammatory mechanisms are considered to play a crucial role (Marshall et al. 1999, DeDooy et al. 2001, Speer 2006). In this thesis no correlation emerged between levels of CD11b expression on circulating phagocytes during the first hours of life and subsequent development of BPD (II, IV). However, in the peripheral blood of infants with RDS, increased proportions of activated T cells during the first days of life correlated with subsequent development of BPD (III). Epidemiological findings in preterm infants demonstrate that risk for BPD is amplified by inflammatory conditions such as infections (Marshall et al. 1999, DeDooy et al. 2001, Speer 2006). Preterm infants with RDS who later develop BPD have during their first two weeks of life a reduced number and increased activation of CD4 cells (Ballabh et al. 2003). Furthermore, accelerated thymic maturation and autoreactive T cells have appeared in baboons with BPD (Rosen et al. 2006). These findings are in accordance with ours and suggest potential involvement of adaptive immune responses in development of BPD.

SYSTEMIC ACTIVATION OF PHAGOCYTES IN INFECTIONS (I)

Postnatal infections in term infants elevate CD11b expression on circulating phagocytes (Nupponen et al. 2001, Weirich et al. 2001). In the preterm infants with proven infection or NEC in Study I, both neutrophil and monocyte CD11b expression levels were significantly higher than in control infants. This increased CD11b expression was already present at the onset of symptoms, which made the clinician order the blood culture test. Furthermore, in at least some infants with infection, neutrophil and monocyte CD11b expression was elevated up to three days before sampling for blood culture, with individual variations (**I:** Table I). This variation in CD11b expression may reflect fluctuation in the clinical course of the evolving septic infection or NEC.

In the present study, both specificity and sensitivity of the CD11b test for late-onset sepsis were reasonably high in monocytes (0.86 and 0.94), but the specificity was quite poor in neutrophils (0.56). To date, at the onset of symptoms clinicians have no reliable means to identify preterm infants with sepsis. Many molecular and soluble markers of inflammation and neutrophil activation have been evaluated as potential sepsis markers in preterm infants (Ng 2004, Ng & Lam 2006, Arnon & Litmanovitz 2008). Unlike acute-phase proteins such as CRP, the CD11b up-regulation does not require protein synthesis (Calafat et al. 1993) and it occurs promptly *in vitro* (Berger et al. 1984, Borregaard et al. 1987) and *in vivo* (Rinder et al. 1992). The CD11b test is well suited as a daily screening test in preterm infants, as the blood volume needed per test is only 25 µl, and the method is reliable with capillary, venous, and arterial sampling. The CD11b test may enable, at least in some infants, early diagnosis of infection and provide means to reduce the use of antimicrobial drugs in neonatal intensive care units. However, our number of patients was low, so results must be interpreted cautiously. In addition, since our findings emerged in an unusual epidemiological setting, the pathogen distribution was uncommon, with as many as three sepsis cases due to *Candida parapsilosis* and only one to *Staphylococcus epidermidis* (**I**: Table I). Therefore, the CD11b test needs to be evaluated further in larger studies under non-epidemic conditions.

METHODOLOGY

The generalizability of our results is influenced by our rather small study-group sizes. Further studies are thus necessary to confirm our results.

In this thesis we used expression of leukocyte adhesion molecules as a marker for systemic inflammation. We chose this strategy because of our earlier experience with CD11b expression on circulating phagocytes in preterm infants. Furthermore, we aimed at keeping blood sample volumes as small as possible. A panel of several different markers combined with soluble markers would, however, have provided a broader view.

In studies II and III, the study groups showed significant differences in gestational age, making it difficult to control the effect of immaturity upon our results. We, however, correlated gestational age with leukocyte activation markers within study groups of infants with and without RDS. In both Studies II and III, in controls the leukocyte activation markers did not correlate with gestational age. In infants with RDS, in Study II CD11b expression correlated negatively with gestational age, and in Study III gestational age correlated negatively with absolute T cell counts, and failed to correlate with CD54 or CD62L expression. Since incidence of both RDS and BPD are highly dependent on gestational age, adjustment for gestational age may attenuate the actual differences between the study groups. The challenge is therefore to distinguish between the effects of pathogenetic processes associated with RDS and BPD and immaturity per se. However, in a physiological context immunoactivation due to pure immaturity is not plausible, whereas immunoactivation in RDS has been shown previously and also makes good sense, biologically.

In Studies III and IV, we analyzed our results with respect to maternal chorioamnionitis, defined clinically as increased maternal CRP >30 mg/l and presence of

clinical signs. However, histologic evaluation of the placentas of mothers with clinical chorioamnionitis and spontaneous delivery could have resulted in more precise diagnostics of chorioamnionitis.

General treatment with glucocorticoids for preterm infants can influence the expression of activation markers on circulating leukocytes. Being aware of this, we used several strategies to control for it. In Study I, infants with infection receiving postnatal hydrocortisone were kept within the study but marked for further evaluation (I: Table 1). In Study II, no infants received postnatal glucocorticoids, and almost all had received antenatal betamethasone (Table 2). In Studies III and IV, the infants receiving postnatal hydrocortisone were excluded after initiation of the treatment, and the timing from initiation of antenatal betamethasone to birth was adjusted for in the analysis.

FUTURE CONSIDERATIONS

Preterm birth exposes the infant to a hostile environment outside the uterus months prior to completion of full gestation. The interrupted uteroplacental supply of oxygen, energy, nutritients, growth factors, and other factors yet undiscovered may have an effect on the infant's immune system as well as on overall development.

Regulation of inflammation in preterm infants may be very different from that in term infants and adults. It would be fascinating to explore in preterm infants the factors leading to polarization of T cell responses to Th1, Th2, and Th17 types and the role of Treg cells. In addition, it would be interesting to study the anti-inflammatory state of preterm infants and its regulation – is stronger inflammation followed by deeper immunosuppression, and does this render the infant susceptible to nosocomial infections?

Current technology enables simultaneous analysis of several soluble cytokines, adhesion molecules, and even genome-wide gene expression in a very small volume of blood. Therefore, the required blood volumes do not limit simultaneous testing of several biological variables. This helps in applying these powerful tools to neonatology and opens new opportunities to study in preterm infants complex biological networks and entire cellular processes from gene transcription and translation to protein-protein interactions.

Just as the lungs may be the crucial organ for acute survival after birth, the brain is the most important organ determining the infant's long-term outcome. Therefore, in future studies, the focus should be on factors influencing brain development and the role of systemic and local inflammation regarding injury to the central nervous system, aiming at better brain protection.

CONCLUSIONS

I. Our results indicate that in preterm infants with birth weight <1000 g, late-onset sepsis is associated with an increase in CD11b expression on neutrophils and monocytes prior to the clinical suspicion of sepsis. These results suggest that daily determination of CD11b expression on circulating phagocytes may prove useful in the earlier diagnosis of late-onset infection. However, this requires larger trials with sufficient numbers of patients to reach definite conclusions.

II. In preterm infants with RDS, the initiation of mechanical ventilation, but not nCPAP treatment or administration of exogenous surfactant, is associated with increased CD11b expression on circulating phagocytes. This activation occurs both in infants treated with prophylactic and in those treated with only rescue surfactant. In preterm infants without ventilatory support, no activation of circulating phagocytes is evident. In preterm infants with RDS, the initiation of mechanical ventilation may be the inducer of the systemic inflammatory response.

III. Systemic activation of T cells takes place during the first postnatal days in preterm infants with RDS, and increased T cell activation is associated with the development of BPD. In preterm infants, priming of the adaptive immunity may lead to an augmented inflammatory response and thereby contribute to the pathogenesis of BPD and to other chronic complications.

IV. In the preterm infant, antenatal exposure to pre-eclampsia, but not to chorioamnionitis, is associated with high systemic activation of inflammation. This may in part be explained by differences in the severity of RDS and lung injury. However, in the preterm infant exposed to pre-eclampsia, intrauterine priming of leukocytes and endothelial activation resulting from placental oxidative stress or insufficient anti-inflammatory mechanisms may also play a role.

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